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THE ORIGIN AND DISTRIBUTION OF
THE ANURA

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THIS paper is a discussion of the former existence of
land bridges from east to west between continents of the
southern hemisphere of the Earth. There are to-day
two north and south intercontinental bridges, the Isth-
mus of Panama connecting North America and South
America, and the Isthmus of Suez connecting Asia
and Africa.

Practically all geologists, paleontologists and biogeog-
raphers agree that during the later Tertiary period there
was eastern and western union of North America with
other continents:—(1) with Europe by way of Greenland,
Iceland, the Faroe Islands and the British Isles; (2) with
Siberia by way of Alaska.

Most paleogeographers—including geologists (empha-
sizing evidence from Earth structure), paleontologists
(emphasizing evidence from fossils) and biogeographers
(emphasizing evidence from the genetic relationships
and geographic distribution of animals and plants) agree
that during the Cretaceous period there was an ocean
channel running westerly and north from the Gulf of
Mexico to the Arctic Ocean, cutting off the westernmost
portion of North America from the rest of the continent,
and that the narrow western land strip so formed was
connected at its northern end with Siberia and at its
southern end with the mountainous region in central

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Central America (fig. 2). Scharff and some others be-
lieve there is sufficient biogeographic evidence to indicate
that the southern end of this east-Pacific land strip was
prolonged to include Ecuador and the Galapagos Islands;
and Scharff, on the basis of a large amount of evidence
from present day animals and plants, carries this land
strip as far south as Chile and Patagonia.

Most paleogeographers now recognize a late Tertiary
connection between Central America and the West In-
dies. Vaughan (1919), for example, judging chiefly from
the present contour of the floor of the Caribbean Sea
and from the geology of southern Central America,
places one bridge between Yucatan and Cuba and a sec-
ond bridge between Honduras and Jamaica.

As to the southern hemisphere opinion is more divided,
but there is a large amount of evidence, accepted by
numerous geologists, paleontologists and biogeograph-
ers, to indicate:—(1) a connection, or connections, be-
tween South America and Australasia (usually regarded
as late Cretaceous or early Tertiary) ; (2) between Africa
and South America—some opinion accepting (*A*) a bridge
from northern Africa to the Guianas and (*B*) a bridge
from South Africa to Patagonia, both these bridges being
usually regarded as Mesozoic, though by some they would
be extended into the earliest Tertiary.

Almost all paleogeographers, if not all, believe that
Australasia was united to the Malaysian islands and the
Asian continent during part of the Mesozoic (Jurassic).

This article is an examination of certain discussions of
the idea of east and west bridges between southern con-
tinents, in which such southern intercontinental connec-
tions have been denied.

Dr. W. D. Matthew, in 1915, published certain theses
as to animal dispersal which may to advantage be studied
critically by the host-parasite method (Metcalf 1921,
1922, 1923). These theses were reiterated by Dr. E. R.
Dunn, last December, at the Boston meeting of the scien-
tific societies and were specifically applied to the Am-

phibia. Let us reexamine these propositions in the light of the dispersal of the Anura and their Opalinid parasites.

Matthew's familiar theses are:—

(1) "Secular climatic change has been an important factor in the evolution of land vertebrates and the principal known cause of their present distribution.

(2) "The principal lines of migration in later geologic epochs have been radial from Holarctic centers of dispersal.

(3) "The geographic changes required to explain the present distribution of land vertebrates are not extensive and for the most part do not affect the permanence of the oceans as defined by the continental shelf.

(4) "The theories of alternation of moist and uniform with arid and zonal climates, as elaborated by Chamberlin, are in exact accord with the course of evolution of land vertebrates, when interpreted with due allowance for the probable gaps in the record.

(5) "The numerous hypothetical land bridges in temperate, tropical and southern regions, connecting continents now separated by deep oceans, which have been advocated by various authors, are improbable and unnecessary to explain geographic distribution. On the contrary the known facts point distinctly to a general permanency of continental outlines during the later epochs of geologic time, provided that due allowance be made for the known or probable gaps in our knowledge.

(6) [p. 180] "Whatever agencies may be assigned as to the cause of the evolution of a race, it should be at first most progressive at the point of original dispersal, and it will continue this progress at that point in response to whatever stimulus originally caused it and spread out in successive waves of migration, each wave higher than the previous one. At any one time, therefore, the most advanced stages should be nearest the center of dispersal, the most conservative stages farthest from it. . . . In fact, it is the environment itself, biotic as well as

physical, that migrates, and the primitive species are those which have followed it, while those that remained have had to adapt themselves to a new environment and become altered thereby.

(7) [p. 311] "The distribution of the Reptilia [intended, apparently, to include Amphibia] appears to be in conformity with the principles here outlined, and extends their application to the Mesozoic era. The distribution of birds and fishes and of invertebrates and plants is probably in accord with the same general principles, modified by differences in methods of dispersal."

Matthew's figure of the distribution of three families of Anura is here given (Fig. 1), corrected for nomenclature and for an inadvertent exchange of family names.
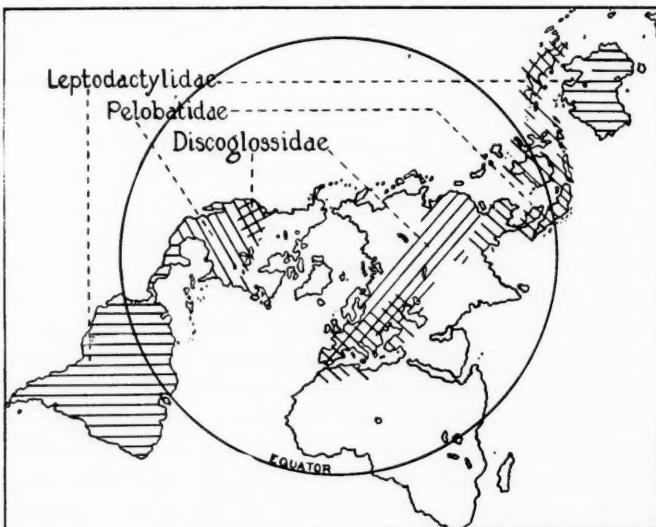

Fig. 1. Distribution of Three Families of Anura, after Matthew (Modified for Nomenclature and for an Inadvertent Exchange of Family Names).

To these theses of Matthew, Dunn (1923) adds [p 135] "The center of dispersal is best determined by the geographic center of the group range."

Matthew's principles of dispersal are attractive, especially his summary of climatic changes and their effects upon faunas and floras.[1] The almost geometric treatment of migration waves (Matthew 6 and Dunn) suggests hesitation and questioning because of the mathematical perfection of the hypothesis. So far as concerns mid-Tertiary and later Mammalia it may well be that Matthew has correctly sketched the broader aspects of their dispersal. Early Tertiary conditions seem doubtful, and the application of his theses to Mesozoic forms seems still more doubtful. We will here review merely part of the evidence from the dispersal of the tailless Amphibia and their Opalinid parasites.

Of the Hylidae Matthew writes: "The Hylidae are to-day chiefly South American and Australian, but a few members *still*[2] inhabit North America. They are not found in Africa [error] or in the oriental region [error] where it seems reasonable to suppose that they have been displaced by the true frogs (Ranidae),[3] peculiarly varied and abundant in these regions." Dunn, having assigned to *Bufo* "dispersal from a northern center," writes: "Similarly *Hyla* is the only genus of Hylidae common to South America and to any of the other continents and it, as does *Bufo,* has a wide northern range." He believes in a northern origin for this family.

The evidence seems to me to point to tropical South American origin of the Hylidae and their spread in two lines of migration: first, westward across the Pacific Ocean to Australia, Papua and the Solomon Islands, not New Zealand,[4] (Fig. 2); and, second, northward to Central America and North America and on by way of an Alaska-Siberia route to Euro-Asia and northern Africa.

[1] Borrowed largely, as he says, from Chamberlin.

[2] Italics mine.

[3] Frogs of the family Ranidae hardly compete with the tree frogs (Hylidae). Their habitats and habits are too different.

[4] As long ago as 1898 Papua was known to have 7 species of *Hyla,* and the Solomon Islands 3. The presence of a Gastrophrynid ["Engystomatid"] (*Calophrynus*) in Samoa and of Limnodynastes, a Leptodactylid, in the New Hebrides is of interest in connection with the hypothesis of a trans-Pacific bridge from South America to Australasia.
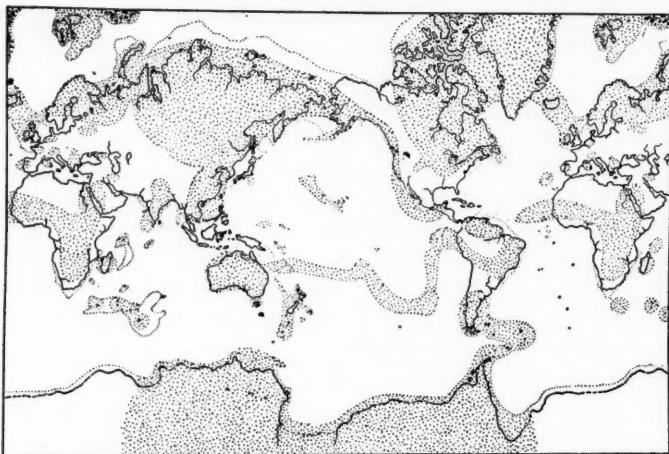
Fig. 2. Relations of Land and Sea at the Close of the Mesozoic or
Beginning of the Tertiary Era.

The trans-Pacific bridge follows the general line of the southern shallows
and includes Samoa (home of *Calophrynus*, a Gastrophrynid) and the New
Hebrides (*Limnodynastes peronii*, an Australian Leptodactylid).   The
Solomon Islands (Hylas 3, Ranas 3, *Cornufer* 2, *Batrachylodes, Cerato-
batrachus,* all endemic) and Papua (*Rana* 3, *Cornufer* 3, *Stenophryne,
Callulops, Xenobatrachus, Xenorhina, Genophryne, Crinia, Hyla* 7, *Ranaster,
Asterophrys*) indicate an early connection with Australia and a later con-
nection with the Malay Islands. They are not united to Australia in this
map, for their union may well have been somewhat later. In a little later
period Australia and New Zealand would be shown united to Antarctica.
Note the land strip from Siberia to Alaska and the western American
coast as far south as the mountains of central Central America. At a little
earlier period this would be shown connected to the south with Ecuador and
the Galapagos Islands. Hawaii (if, as is improbable, *Bufo dialopus* be a
native species), probably, then, had at about this time an Arctogean con-
nection by which its *Bufo* entered. That this was to the west, if it existed,
is probable in view of the fact that the western islands of the group are the
older, the group having been advancing to the eastward by the rising of
new land at the eastern end of the group and the subsidence of land to the
west, the movement of the group as a whole being like that of a wave. This
eastward movement is said now to have ceased.
The connection of Patagonia to Antarctica may have disappeared before
the Antarctic union with Australia and New Zealand was established, mak-
ing here a similar land wave moving from east to west. There is some
evidence, not conclusive, that the migration of animals at about this time
was westward rather than eastward between America and Australia.
The earlier (Jurassic and early Cretaceous) connection between Patagonia
and southern Africa is not shown, and the union of northern Africa and the
Guianas is disappearing. There is perhaps some indication, emphasized by
some zoögeographers, that the Africa-South America connection or connec-
tions may have persisted into the early Tertiary.
[Map compiled, with modifications, from Arldt, Schuchert, von Ihering,
Scharff and Haug.]

The several genera of the Hylidae are very similar to one another; *Chorophilus, Acris* and *Agalychnis* might well be regarded as subgenera of the genus *Hyla.*

The Hylidae and the Leptodactylidae ("Cystignathidae") are very near relatives and in all likelihood arose from common ancestors. The Leptodactylidae now occur in tropical America, New Zealand (*Liopelma,*[5] 2 species), Tasmania, Australia, Papua, the New Hebrides (the Australian species *Limnodynastes peronii*). The Hylidae (Fig. 3) are abundant in tropical America and Australia,
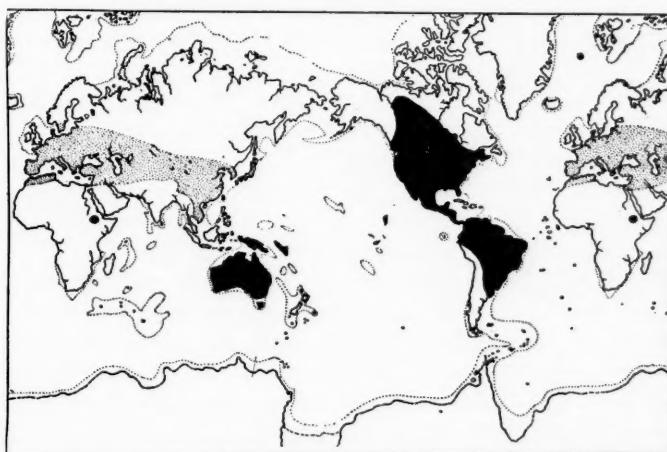

FIG. 3. DISTRIBUTION OF HYLIDAE.

The stippled areas are occupied only by *Hyla arborea* and about half a dozen closely related forms, perhaps best treated as subspecies. The status of "*Hyla wachei*" from Abyssinia is still uncertain.

are well represented in Tasmania, Papua (7 +) and the Solomon Islands (3), but not in New Zealand, are present (1 species, *Hyla arborea chinensis*) in Formosa (Vogt, 1911), are abundant in North America and a few forms (probably best regarded as offshoots from a single immigrant species, perhaps as one species with half a dozen or so subspecies) are found in Euro-Asia (including eastern and southeastern Asia, Formosa, India, the

[5] First described as a Discoglossid.

Caucasus, Asia Minor, Europe), in northernmost Africa and in Abyssinia.[6] Fossil Hylids or Leptodactylids have not been reported, so far as the author knows.

The presence of even a few Hylids in Euro-Asia seems to give comfort to the advocate of a northern origin of this family, rendering it, at first thought, more likely that the Australasian Hylids may have entered their present habitat from the north by way of Malaysia. There are, however, definite difficulties in the way of such a hypothesis.

(1) The Euro-Asian Hylids are much more nearly related to North American species than they are to Australasian species.

(2) The Opalinid parasites of the Euro-Asian Hylids,[7] so far as known, are of a modern subgenus (*Opalinae angustae,* evolved, apparently, in the Pliocene), are of North American origin (Fig. 4), and are utterly different from any Opalinids known in Australasia. *Opalina obtrigona* was the Opalinid found in all the infected Hylas from Euro-Asia. This is perhaps the most modern of all Opalinidae. The Australian Hylids, on the other hand, have been found to carry Opalinids only of the most archaic genus, *Protoopalina,* a genus of world-wide distribution. The Euro-Asian Hylid parasite, *Opalina obtrigona,* is almost identical with the North American species *O. obtrigonoidea,* which occurs in several North American genera, including Hylids of five species.

A few words as to the classification and occurrence of the Opalinidae will emphasize the evidence they give as to the origin and dispersal of their hosts. In the family Opalinidae are two subfamilies and four genera, as follows:

[6] The reference of this Abyssinian form to the family Hylidae has been questioned.

[7] But three Palearctic Hylas have been successfully searched for Opalinids —*Hyla arborea* from western Europe, *H. arborea savignyi* from Jerusalem and *H. arborea japonica* from Japan. One individual each of three other subspecies (*chinensis, meridionalis* and *stepheni*) have been opened but found uninfected.
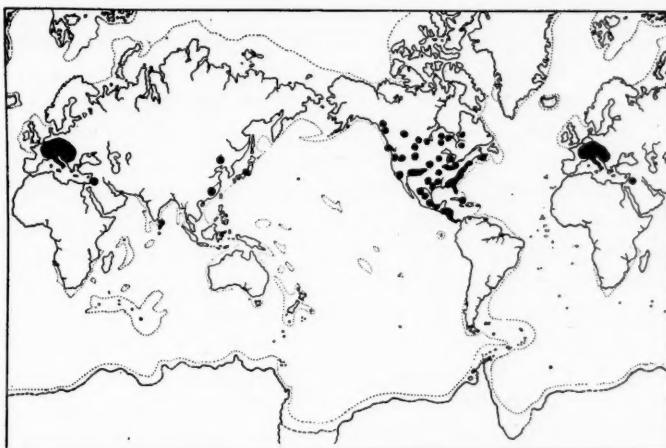
FIG. 4. DISTRIBUTION OF THE OPALINAE ANGUSTAE.

The genetic relationship of the Ceylonese *Opalina virgula* to this subgenus is doubtful, because of both its structure and its geographical position. All other Palearctic records shown are of *Opalina obtrigona* only.

Opalinidae
> *Protoopalininae* (2 nuclei)
>> *Protoopalina* (cylindrical, or at least not much flattened), 9 subgenera
> *Zelleriella* (much flattened)
> *Opalininae* (multinucleated)
>> *Opalinae latae* (broad forms of oriental origin)
>> *Opalinae angustae* (narrow forms of Nearctic origin).

The indications are strong that *Zelleriella* arose in tropical America from *Protoopalina*; that *Cepedea* evolved from *Protoopalina*, probably in southern Asia; that *Opalina* (broad form) was derived from *Cepedea*, apparently in Euro-Asia; that the *Opalinae angustae* arose in southwestern North America or in Central America when Hylids, coming north from South America, first met Bufos and Ranas bearing broad *Opalinae*, adopted these parasites and changed them to the narrow form. The change of form of the *Opalinae* in Hylid hosts

occurred probably after the middle Pliocene, when the
Isthmus of Panama was definitely formed (Vaughan,
1919), though it is possible that Hylids entered Central
America and western North America earlier in the Ter-
tiary by way of a land strip somewhat to the westward of
the present Isthmus of Panama (Fig. 2). The presence
of broad *Opalinae* in America before the later Tertiary
is, however, improbable, the genus *Opalina* being ap-
parently of late Tertiary origin in Asia.[8] Narrow *Opa-
linae* are found only in North America and Central Amer-
ica, except for the one species, *O. obtrigona,* in the Euro-
Asian *Hyla arborea* and its subspecies. It seems pretty
clear that *Hyla arborea,* or its immediate ancestor, very
closely related to western North American species,
passed from North America by the Alaska-Siberia route,
across Euro-Asia and into northern Africa, carrying
with it the North American narrow *Opalina* which now
discloses the place of origin of its host. It seems equally
clear that the Australasian Hylas did not come from
Euro-Asia, for there are no Euro-Asian Hylas known
to-day except those of close North American affinities
and we know no Palearctic fossil Hylas. It seems wholly
unlikely that Hylids, whether of Nearctic or of Palearctic
origin, could have entered Australasia from the north
without leaving in Euro-Asia and Malaysia species show-
ing affinity to present Australasian tree frogs.

No multinucleated Opalinids are known from Austra-
lasia. If the Australian Hylas had entered from Asia
they should have brought multinucleated Cepedeas or
Opalinas, such as are found in Nearctic Hylids, unless
they left Asia before the evolution and presence there of
these multinucleated genera. All the indications seem
distinctly against the hypothesis of the Australasian
Hylids having entered Australasia from the north.

The Leptodactylidae present equally difficult phenom-
ena to the one who seeks to explain their present distribu-
tion upon the theory of northern origin (Fig. 5). They

[8] For fuller discussion of these relations see Metcalf (1923).
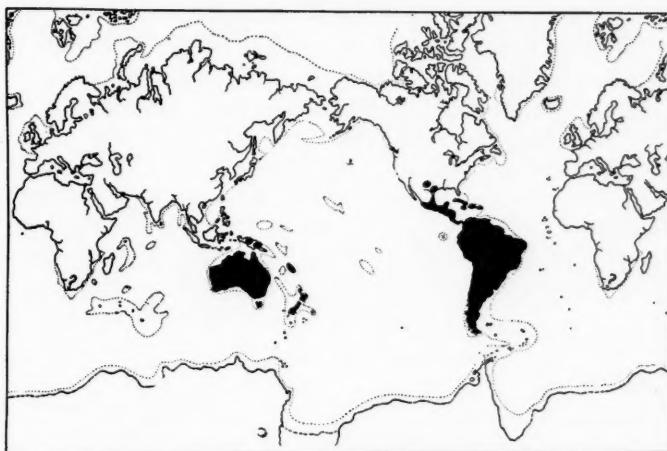
FIG. 5. DISTRIBUTION OF LEPTODACTYLIDAE.

The question mark in southern Africa indicates doubt that has been expressed as to the genetic relationship of its genus *Heleophryne* to the Leptodactylids.

are to-day confined to tropical America (ca. 250 species), New Zealand (*Liopelma*, 2 species), Tasmania (4 species), Australia (ca. 30 species), Papua (*Genophryne* and *Crinia* and perhaps others of recent record) and the New Hebrides Islands (*Limnodynastes peronii*, also in Australia), with the possible addition of southern Africa, from which region *Heleophryne* (2 species) has been reported. The American and Australian Leptodactylids bear parasitic Zelleriellas so similar that they may be specifically identical, and the genus *Zelleriella* (Fig. 6) is known only from tropical America and its North American fringes and from Australia.[9] If the Leptodactylids

[9] Bezzenberger's (1904) report of a *Zelleriella* (*macronucleata*) from "Asia" is so vague as to locality, and the likelihood of confusion as to locality is so considerable, that this species can not be taken into account. The species may be identical with an Australian or tropical American species or both. See Metcalf (1923). Recent as yet uncompleted studies of certain Cepedeas, made by a friend, and to which it is not appropriate now to refer more fully, cast grave doubt upon *Zelleriella macronucleata* being an adult form or even a *Zelleriella* at all. The occurrence of this Opalinid in "Asia" gives no evidence upon the question here under discussion.
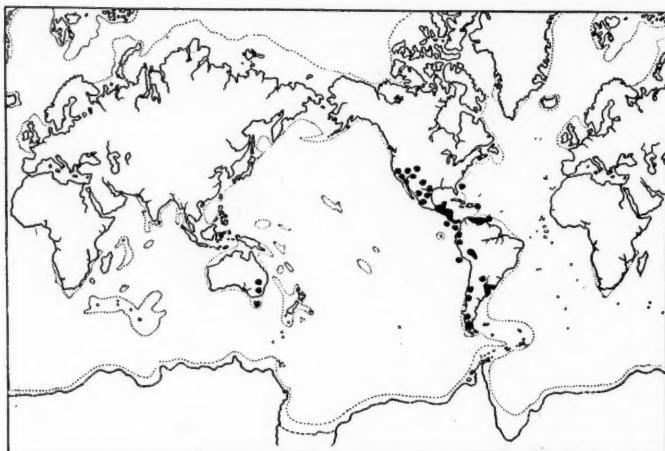
FIG. 6.   DISTRIBUTION OF ZELLERIELLA.

arose in the north and spread southward to South America and Australasia, how is it that no Leptodactylids or Zelleriellas are found in Euro-Asia or in north-temperate North America?[10]   In tropical America and in temperate South America and Australia the Leptodactylids are vigorous dominant forms.   If Leptodactylids, indeed, were once in Asia and have become extinct there or been driven out, their *Zelleriella* parasites should now be found in the Asian Bufos, for toads readily adopt any Opalinids with which they come into contact.   Leptodactylids endure well the cold of Patagonia and of the high Andes, so they can not well have been driven out of Euro-Asia by inclement climate.   There seem no indications that they ever were in Palearctica, and they have now reached only the southern fringes of the Nearctic region.   Unless further search reveals Leptodactylids or Zelleriellas or both in Asia, there seems scant possibility of the former presence of Leptodactylids in Asia.   *Zelleriella* seems a comparatively modern genus, probably of early or middle Tertiary origin, and its spread from South America to Central America and North America

10 No fossil Leptodactylids are known from Arctogea.

was probably in Pliocene times, though it may possibly have reached these northern lands earlier, by a land route lying to the west of the Isthmus of Panama (Fig. 2), at a time before the present Isthmus was formed.

We should note also that probably the most archaic subgenus of the genus *Protoopalina* (Fig. 7) is represented in Australian and Patagonian Leptodactylids and that Johnston (1912, 1914) reports the Anuran Trematoda of Australia as showing far closer affinities with those of South America than with those of any other region.
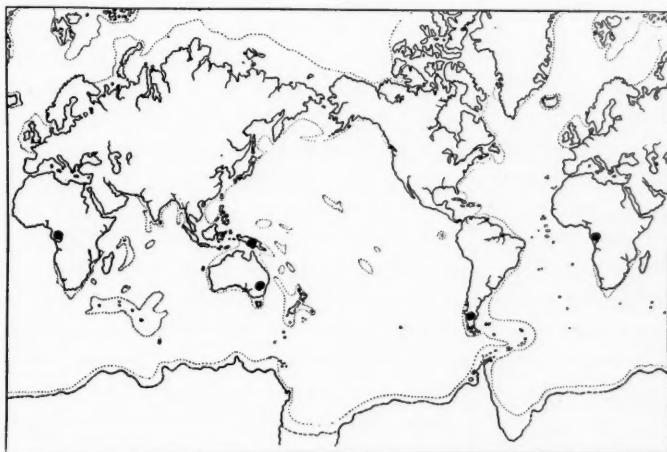
FIG. 7. DISTRIBUTION OF THE MOST ARCHAIC SUBGENUS OF PROTOOPALINA.

The evidence seems clear that the Leptodactylids arose in South America and that *Zelleriella* evolved in these South American hosts. Also the evidence seems strong that neither Leptodactylids nor Zelleriellas have ever lived in Asia. How then did Australasia (including New Zealand and Papuasia) get its Leptodactylids and their parasites the Zelleriellas? The answer seems of necessity to be—by some southern route. And the distribution of the Hylidae also calls for a southern route between tropical America and Australasia, there being no pale-

arctic Hylids except such as by their genetic relation-
ships and their parasites are shown to have emigrated
very recently from North America.

The New Zealand Liopelmas were described as Disco-
glossids, but further study has now led to their classifi-
cation among the Leptodactylids.

What shall we say of *Heleophryne* in South Africa?
This form has an arciferous sternum and has been
classed as a Leptodactylid. The suggestion has, how-
ever, been made that it may be genetically a Ranid which
has not completed its development from the arciferous to
the firmisternous condition. If it be genetically a Lep-
todactylid, it would seem to indicate the origin of the
Leptodactylidae at or before a period when southern
South America and southern Africa were connected by a
trans-Atlantic bridge—often called Archiplata. This
southern trans-Atlantic bridge is usually regarded as
having become permanently interrupted in the Creta-
ceous period, though by some it has been thought to have
persisted into the Tertiary. If the African *Heleophryne*
proves to be a Leptodactylid, it would tend to date the
origin of this family somewhat earlier geologically than
the South America-Australia evidence would necessitate.
If *Heleophryne* should be found to bear *Zelleriella* this
would seem evidence, difficult to combat, that South
Africa and South America were once united by a south-
ern migration route.

The Hylidae and the Leptodactylidae are among the
more modern families of Anura, possibly more modern
than any others of the usually recognized families,
though the Ranidae are in some respects structurally
more developed. If these comparatively modern forms
do not fit into Matthew's schema of dispersal, it is likely
some of the other families may not do so.

The present distribution of the genus *Rana* (Fig. 8)
and a study of its Opalinid parasites indicates a probable
northern origin of this genus. Except for a single species
in northern South America, several species in Papua, one
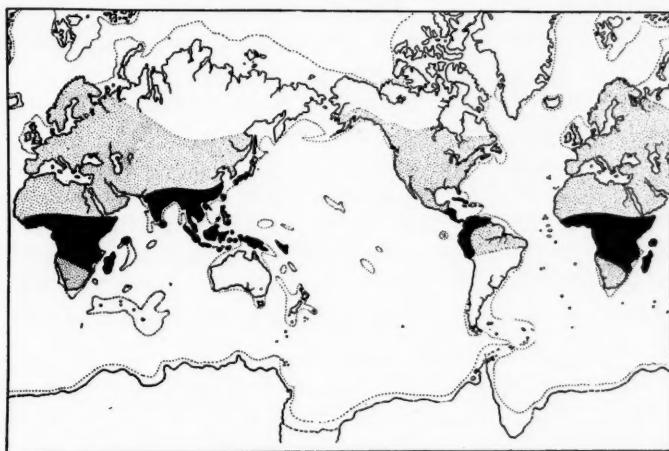
FIG. 8. DISTRIBUTION OF RANINAE.
The presence of genera other than *Rana* is indicated in black. *Rana* is found over both the stippled and the black areas except that it is absent from Madagascar and the Seychelles Islands.

Papuan species in the northernmost tip of Australia, and three species in the Solomon Islands, they have not penetrated into the southern hemisphere except in Malaysia and in Africa. Their entrance into the Malaysian Islands and Papuasia was probably in the late Tertiary, and their entrance into tropical Africa may well have been by way of the Nile valley, this also in later Tertiary times. Fossil Ranas are known from European rocks of the lower Miocene and perhaps of the upper Oligocene. Other genera of the same subfamily, *Raninae,* have an Equatorial distribution and we seem to find nothing in themselves, their fossil remains,[11] or their Opalinids, to determine definitely whether their original habitat was in "Equatorian" lands or was northern. The former has seemed to me the more probable (Metcalf, 1923).

[11] The reported presence of *Oxyglossus* in the Eocene of Wyoming is difficult to accept, at least without a very thorough review of both the biotic and geologic evidence. This seems, indeed, to be the same form which Moodie (1914) describes from Marsh's specimens as *Eobatrachus agilis* Marsh. Moodie regards it as probably a Bufonid, and he assigns it to the Jurassic. It may be lower Cretaceous.

The *Dendrobatinae* (Fig. 9), another subfamily of the Ranidae, are of exclusively southern habitat to-day and I know of nothing in themselves or in their Opalinid parasites to suggest migration at any time southward from a former Arctogean home.

The Ranid *Ceratobatrachus,* the sole representative of the *Ceratobatrachinae,* is known only from the Solomon Islands and apparently throws little light upon the hypotheses under discussion. The Solomon Islands have strong Papuan affinities in their Anuran fauna. There is zoogeographic evidence of Papua having had Malay-
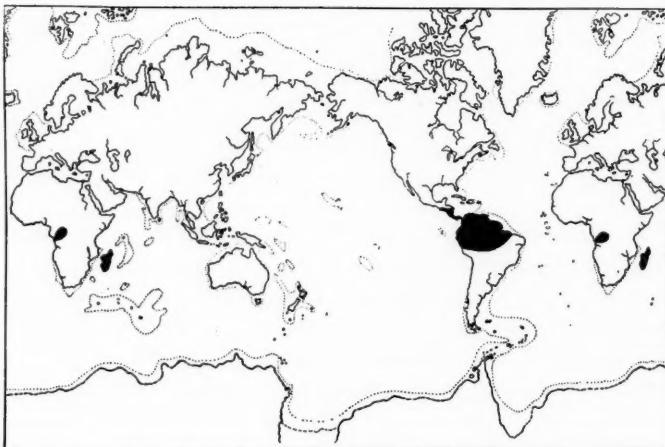
Fig. 9.   Distribution of Dendrobatinae.

sian connections since it separated from Australia. The development of a subfamily of Ranids (*Ceratobatrachinae*) exclusively in the Solomon Islands, and the fact that all the Anura in the islands (9 species of 3 families) are endemic, makes probable an early separation of these islands from Papua, though subsequent to the separation of Papua from Australia.

The Gastrophrynidae ["Engystomatidae"] (Fig. 10), another firmisternous family and probably comparatively modern, are to-day confined to the tropics and to
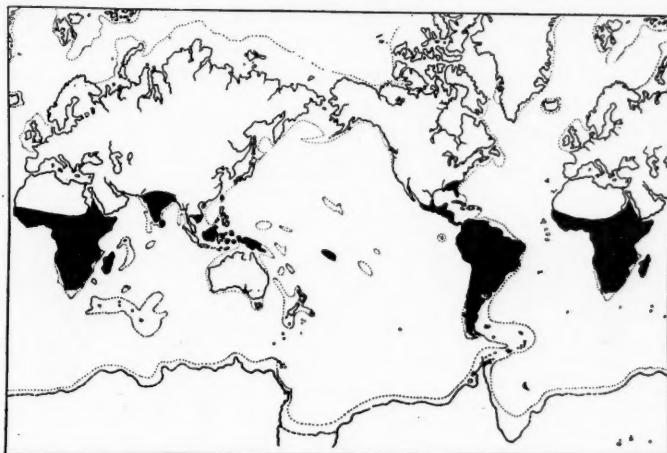
FIG. 10. DISTRIBUTION OF GASTROPHRYNIDAE.

The distribution of the genus *Calophrynus* (Madagascar, Burma, Borneo, Samoa) is of especial interest.

south-temperate lands, except for representation in India, southern China, Formosa and southeastern United States. They are hospitable to all genera of Opalinids, evidently adopting in any locality the Opalinids there present. I know of nothing in themselves or in their parasites to indicate northern origin and we have no significant data from fossil forms. The fact that two Gastrophrynids bear Opalinids apparently transitional between the genera *Protoopalina* and *Zelleriella* is an indication, of not too great weight, that they have lived for a goodly period in tropical America, the place of origin of the genus *Zelleriella*. The presence of a Gastrophrynid (*Calophrynus*) in Samoa and of an Australian Leptodactylid (*Limnodynastes*) in the New Hebrides is worthy of notice. The latter probably migrated eastward to its present island home. The Samoan Gastrophrynid may likewise have entered from the west.

Of the Bufonidae, the probably more modern genus *Bufo* (Fig. 11) is cosmopolitan, except for Australasia and Madagascar, one species (*Bufo dialopus*) being re-

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ported even from the Hawaiian Islands, though its being
a native form has been questioned, the other, more ar-
chaic, genera (Fig. 12) are exclusively tropical. I know
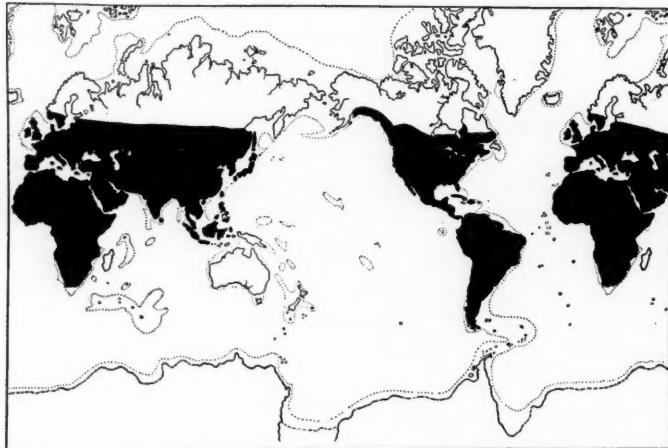

FIG. 11. DISTRIBUTION OF BUFO.
The report of a native *Bufo* (*dialopus*) in the Hawaiian Islands has been
questioned.
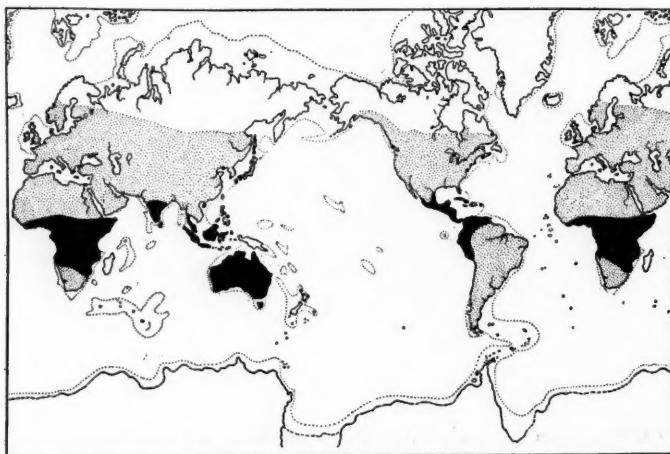
FIG. 12. DISTRIBUTION OF BUFONIDAE.
The presence of archaic genera is indicated in black. *Bufo* is found over
all the stippled and the black areas except Australia and Tasmania. The
report of a native *Bufo* (*dialopus*) in Hawaii is questioned.

of no indication that these more archaic Bufonids may have had a northern origin.[12] Fossil Bufonids are known from the early Tertiary of Arctogea, but these apparently are all referable to the genus *Bufo*. The Bufonidae, then, give no evidence in favor of Matthew's hypothesis. No more do their Opalinid parasites, for *Bufo* adopts any Opalinids present in its habitat, and other Bufonid genera may likely have the same habit. It is of interest to note that none of the Bufonidae of South America are found to carry any multinucleated Opalinids, though these occur in the Bufos of Central America and southwestern North America, indicating that probably Bufonids have not passed southward over the Isthmus of Panama since the entrance of the genera *Cepedea* and *Opalina* into southwestern Nearctica.
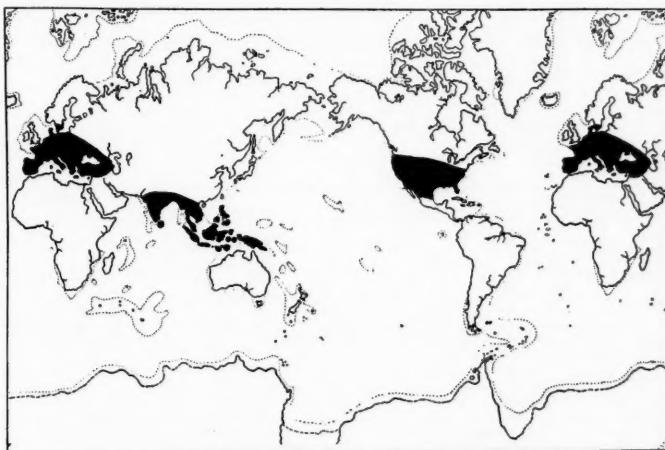
FIG. 13. DISTRIBUTION OF PELOBATIDAE.
The American Pelobatids are all of the exclusively American genus *Scaphiopus* ("Spade-foot Toads").

The Pelobatidae (Fig. 13), perhaps an offshoot from early ancestors of the Bufonidae, are to-day exclusively Arctogean, except that they have spread across Malay-

[12] *Eobatrachus agilis* Marsh is regarded doubtfully by Moodie (1912, 1914) as a Bufonid, possibly of the genus *Bufo*, but until its relationships are better understood it can hardly be taken into account.

sia to Papua. Their Opalinids are of the archaic genus *Protoopalina,* except that in North America the endemic Pelobatid genus *Scaphiopus* has adopted also one *Zelleriella,* one, or possibly two *Cepedeae* and two modern narrow *Opalinae.* I have suggested the origin of the Pelobatidae in southeastern Asia soon after the separation of Australia from Asia at the beginning of the Cretaceous period (Metcalf, 1923).
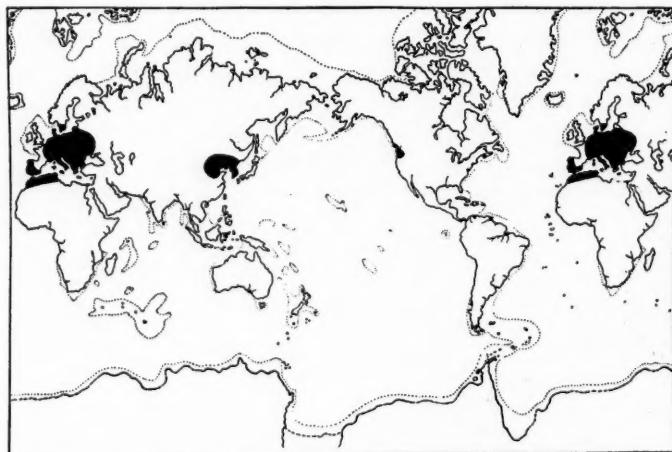
Fig. 14. Distribution of Discoglossidae.
On the western coast of North America, in the region near Puget Sound, is found *Ascaphus truei,* the only Discoglossid of the western hemisphere.

The Discoglossidae (Fig. 14) are northern to-day and I know of no indication in themselves or in their parasites to indicate the presence of members of this family at any time in the southern hemisphere. *Liopelma,* represented to-day by two species in New Zealand, was first described as a Discoglossid, but further study has resulted in assigning this genus to the family Leptodactylidae.

The Pipidae (Fig. 15) are probably among the more archaic of the Anura. They are now confined to the tropics, one subfamily, the *Xenopodinae,* in southern and
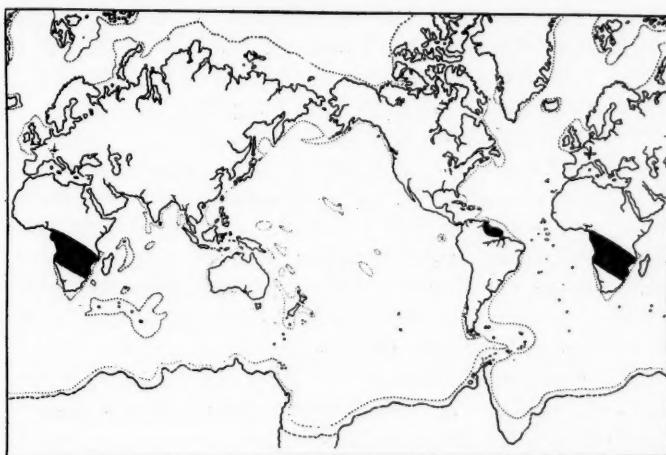
FIG. 15. DISTRIBUTION OF PIPIDAE.

In Africa *Xenopus* (2 species) and *Hymenochirus;* in South America *Pipa.* The presence of fossil *Palaeobatrachus* in Europe is indicated by a cross.

western Africa, and another subfamily, the *Pipinae,* in northern South America. Their known Opalinids are Protoopalinas of an archaic species reported only from Africa. One genus of Pipids, *Paleobatrachus,* was apparently abundant in Europe in Miocene times. The family arose probably several geologic periods earlier than this, for the Anura, from their present distribution, seem to have evolved at least as early as the Triassic period (Metcalf, 1923). If we regard *Pelion lyelli* as ancestral to the Anura, their evolution had begun in the Carboniferous period (mid-Pennsylvanian, Linton beds, of Ohio).[13] The extent of the distribution of the earliest Anura in pre-Triassic times is not indicated by fossil remains. Indeed we have no undisputed pre-Cretaceous remains of Anura.

Subfamilies whose parasites are unknown are not included in this discussion.

What, then, are the suggestions from this review of data?

[13] See Moodie (1914).

(1) The Anura seem to have arisen in the earliest Mesozoic, or even in the Paleozoic era, but the extent of their dispersal in these early times is not indicated by evidence from themselves or their parasites (except in connection with paleogeographic data still *sub judice*).

(2) Some groups of Anura are now wholly or chiefly of northern habitat and may well always have been northern. Among these are the Archaic Discoglossidae, the Pelobatidae (a far from modern family) and the *Raninae* (a subfamily highly evolved and structurally comparatively modern, though fossil Ranas are known from strata as early as the Oligocene).

(3) One ancient group of Anura, the Pipidae, is now of southern habitat, but shows evidence from fossils of having once been also in the north.

(4) Other groups are now chiefly southern with some representatives in the southern edges of some Arctogean lands: Bufonidae (other than *Bufo*), Gastrophrynidae, *Raninae* (other than *Rana*), *Dendrobatinae,* Leptodactylidae. The southern genera of Bufonidae seem more archaic than *Bufo,* and the southern genera of *Raninae* seem more archaic than *Rana.*

(5) The Leptodactylidae, while well represented in Central America and present in Texas and the West Indies, are a southern family. They carry a southern Opalinid parasite (*Zelleriella*), and they seem never to have been in Euro-Asia, though found to-day in Australia, Tasmania, New Zealand, Papua and Erromango (one of the New Hebrides Islands). Their route of transfer between South America and Australasia seems to have been southern—probably by way of Antarctica during its period of mild moist climate in the early Miocene.

(6) The Hylidae, though represented to-day in Euro-Asia by half a dozen forms (perhaps one species with several subspecies) carrying modern Opalinid parasites of North American origin, are an American and Australasian family, apparently evolved in South America. Their route of migration between tropical America and

Australasia seems to have been southern, apparently across the Pacific Ocean and not by way of Antarctica, for they are not found to-day in southern South America or in New Zealand. The Samoan Gastrophrynid may have travelled by this same route, but whether eastward or westward is not indicated.

How do these data and conclusions fit with Matthew's and Dunn's theses?

Matthew No. 1 and No. 4: importance of climatic change as an influence upon distribution—not affected.

Matthew No. 2 and No. 7: principal lines of migration in later (including Mesozoic) geologic epochs radial from Holarctic centers of dispersal—opposed by the Hylidae and Leptodactylidae and their Opalinid and Trematode parasites.

Matthew No. 3 and No. 5: permanence of present oceans and no transoceanic bridges—opposed by the Hylidae and Leptodactylidae and their Opalinid and Trematode parasites.

Matthew No. 6: successive waves of migration from an evolution center, the more modern forms pushing the older forms toward the periphery—not supported by any of the Anura or their parasites, and opposed by some of the Anura. Note especially *Ascaphus truei* (Fig. 14), an ancient discoglossid, still present in the Olympic and Siskiyou Mountains, part of a generally accepted land strip between Asia and North America (Fig. 2). Instead of the more vigorous, modern forms pushing out the less vigorous, older forms, it seems that the more vigorous genera themselves spread over wide areas (*Rana, Bufo, Hyla*), while the less vigorous, perhaps diminishing in numbers, persist in their old habitats, in some cases aided by protective habits of seclusion (as, for example, the genera *Ascaphus* and *Scaphiopus* in North America, both sluggish species seemingly peculiarly defenceless), or by such special adaptations as the peculiarly disagreeable skin secretions of the Euro-Asian Discoglossid *Bombina* ("*Bombinator*").

In brief summary I would say that dispersal of land animals southward from Holarctic regions, especially into Africa, seems to have obtained in the later Tertiary, a period for which no southern intercontinental land bridges have been postulated. It also seems evident that northward migration from South America took place in the latest Tertiary. Whether there were east and west migration routes between southern continents during the early Tertiary depends upon the date of such bridges. That they existed at some time seems indicated by the biogeographic evidence. When they existed, whether in Paleozoic and Mesozoic times only, or also in the early Tertiary, is still a mooted question.

Dunn's statement that the center of dispersal is best determined by the geographic center of the group range is too geometrical and too little biological, and could hardly apply to any family of Anura. Other data not conflicting, the region in which a family or subfamily is represented by the greatest number and variety of genera, or a genus by the greatest number and variety of species, is likely to have been inhabited by the group for a longer period than a region in which the group in question shows less divergent differentiation. But this latter suggestion does not seem to hold true without exception. Note the Hylidae, a family especially well adapted to the moist forests of tropical America. The largest number of its genera (subgenera) are in North America where the members of this tropical forest-loving family have spread to a region of greater variety of environment both climatic and physiographic. Animals are exposed to so varied and so intricate influences that their distribution can hardly be expressed upon geometric principles.

In all the above discussion I have followed the usually accepted conceptions of relationship among the Anura rather than Noble's (1922) more recent classification based partly upon the skeleton but chiefly upon the thigh musculature. I do not wish to express, nor even to exer-

cise, independent judgment as to the general validity of Noble's classification, preferring to await further reaction from special students of the Anura. My only comment would be that Noble makes considerable and repeated use of the conception of parallel evolution in the cases of resemblance between animals living in widely separated habitats between which migration by routes available at present would be difficult to conceive. The outworking of parallel inherent tendencies in the evolution of divergent branches of certain Protozoan stocks (for example, the Ciliate parasites in the rumen of the Ungulata) seems clearly indicated, and in higher groups there are instances of parallel development of nearly related branches, but the conception is one to be invoked only with great caution and when the evidence from all sources seems quite clear. Its application to modern representatives of a conservative group of probably Paleozoic origin and of Paleozoic or Mesozoic differentiation calls for especially thorough scrutiny.

But whatever the result of further consideration of Noble's general taxonomic system, I find it peculiarly difficult to believe that, for example, the Leptodactylidae of tropical America and those of Australasia are not closely related genetically; and that similarly the *Zelleriella* parasites of the American Leptodactylids, almost, if not quite, specifically identical with the Zelleriellas of Australian Leptodactylids, are also the result of parallel evolution in the two continents; and that, at the same time, the Leptodactylid Trematoda of the two continents are closely similar because of a third series of parallel evolutions in these two regions of the Earth.

The possible alternatives also seem very difficult to accept:—either (1), *a*, of pre-Cretaceous presence in Arctogea of Leptodactylids, Zelleriellas and the special Trematoda characteristic of Australian and South American Leptodactylids and Hylids, *b*, of their pre-Cretaceous or early Cretaceous migration to Australasia and

the American tropics,[14] and, c, of their complete suppression in the northern lands; or (2), a, of a later evolution of the three sets of forms, b, a late Tertiary migration to their southern homes, and, c, a complete disappearance from the north since, say, the middle Pliocene, the time of the establishment of the Isthmus of Panama (Vaughan, 1919).

The host-parasite evidence seems to me unassailable; multiple parallel evolution is not to be considered seriously. And the alternative assumptions, if we accept the host-parasite evidence and reject the idea of east and west migration routes in the southern hemisphere, seem more difficult than the hypothesis of former transoceanic bridges. It is somewhat interesting to note the somewhat general agreement of geologists, paleontologists and biogeographers as to the existence, general position and time of such intercontinental connections.

But perhaps the chief point of interest in such discussion as this is not any particular conclusion or any series of conclusions, but is, rather, the indispensable assistance of the host-parasite method in attack upon all such, and many other, problems. The host-parasite mine must be worked for all groups of animals and plants. Geologists, paleontologists, biogeographers, taxonomists and parasitologists must cooperate in the study of these problems, and the contribution of the parasitologists will not be the least important.

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THE BEHAVIOR OF BLOOD-SUGAR VALUES IN HEREDITY

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CARNEGIE STATION FOR EXPERIMENTAL EVOLUTION

THE behavior in crosses of a character similar to or comparable with the normal sugar level of the blood has hitherto not been investigated. Earlier physiological work indicates that this is a character which is normally maintained at a given level throughout the whole of the life cycle so far as this has been studied; probably it is normally essentially constant for the species or race; in the individual it is much modified in certain diseases, and it shows very wide temporary fluctuations under a variety of external and internal conditions; finally, changes in blood sugar concentration are necessarily accompanied by other changes of concentration or of equilibrium not only in the blood itself but in the living tissues which must in turn equilibrate or adjust to changes in this circulating tissue. If, therefore, from a cross of different species or genera, a *hybrid* with a definitely new or different sugar level could be formed, all this hybrid's characteristics would be forced to undergo practically the whole of their development in an intimately surrounding medium different from that of its parents. Possibly we should thus have an opportunity to observe the response of the parental characters to this change (and doubtless of course to some or many other unknown or unstudied simultaneous changes) in the blood.

Studies on the inheritance of visible characters in crosses of widely separated forms—of distinct species, genera or families—have shown in some cases (Whitman, 15) marked irregularities and behavior of characters elsewhere known to segregate and show complete dominance or complete recessiveness. In other cases simi-

larly wide crosses of other biological types have apparently failed to show notable departures from the expected behavior of these same characters. To us it seems probable that the results of the present study throw some light on the reason for the aberrant behavior of these visible characters in some generic crosses and also for the normal behavior of these same characters in other generic crosses. Whether justified in this speculation or not we are now able to establish one or two facts concerning the hereditary behavior of blood sugar values in two pairs of generic crosses.

The possibility of studying the behavior of blood sugar values in inheritance has only recently become apparent. This possibility has arisen in part through the fuller recognition and partial elimination of several factors which cause wide temporary fluctuations in the sugar values obtained from pigeons (6, 9, 10, 11, 12) ; and more important still from our demonstration (6, 9, 10) that different genera and families of pigeons—previously known to be fertile in crosses—have distinctly different normal levels of sugar in their blood. A fortunate aspect of those results presents itself in the circumstance that the two species of the two genera which previously had been most extensively used in genetic and sex studies by one of us were there shown to have almost the highest (*T. orientalis*; see Table II) and quite the lowest (*St. alba*) blood sugar values obtained from the several genera examined. And from this cross a sufficient number of F_1 hybrids and of F_1 back-crossed with one parent species were at hand for a partial investigation of this problem. From still another generic cross the two parents and 37 of their offspring supplied material for a second test.

MATERIALS AND METHODS

After other studies had made it clear that, of the several species of pigeons studied by us, the Japanese turtle dove (*Turtur orientalis*) has almost the highest concen-

tration of blood sugar and the ring doves (*Streptopelia alba* and *St. risoria*) have the lowest, it was decided to use all the adult hybrids of this cross then in our collection as the chief material of the present study. These hybrids were aged 17 to 183 months. From the 81 F_1 hybrids taken for study we obtained duplicate sugar determinations on 38 individuals which proved to be free of observable disease at autopsy. All the birds used in the study were killed after drawing the last blood sample to discover possible disease and to make sure that no ovulations were imminent in the females (6, 9). For hybrids other than the F_1 the available number of healthy individuals was considerably less—only 7 were "five eighths" Turtur hybrids; 8 were "one fourth" Turtur; 8 were either "one thirty second" or "one sixty fourth" Turtur and these are combined here and treated as one group. A study of a group of "one eighth" Turtur hybrids was delayed until after the onset of cold weather (October 19). Before this work could be carried out other studies had shown (11) that the blood sugar is considerably affected by the onset of cold weather in the autumn and the proposed study of this group of *Turtur* hybrids was therefore abandoned. The 10 *Turtur orientalis* and the 10 *Streptopelia* listed in Table I as "parent species" (all aged 10 to 47 months) are in most cases not the actual parents of any hybrids represented in the table; these hybrids arose from numerous matings of individuals of these genera, and in most cases the parents of the hybrids were not alive at the time this study was begun. The sugar determinations for the *Turtur* and *Streptopelia* "parents" and for the several groups of their hybrids were all made between April 28 and October 15. Except for a very few individuals this period ended on August 17.

A second group of F_1 generic hybrids—a large single fraternity from a cross of a *Spilopelia* male with a *Streptopelia* female—was also studied; and in this case the sugar values of the actual parents and of the frater-

nity of the female parent were determined. Autopsy showed, however, that only 20 of the 37 F_1 individuals from this generic cross were healthy; there being 7 tuberculous birds and 10 others infested with round worms. Several of these diseased birds gave very aberrant sugar values. The male *Spilopelia* parent was an imported (tropical) bird of uncertain age but certainly more than 3 years old and fully fertile in this generic cross. The female parent was 25 months old. Their hybrid offspring were all hatched in the same year and were aged 6 to 16 months when taken for this study. All the sugar determinations for all members of this group of birds were made between June 17 and August 17.

All the blood samples (except 12 *Streptopelia* and 12 *Turtur,* included in Figure 2, from samples obtained from the upper beak) were drawn by needle-puncture of the heart, and duplicate samples were taken usually at intervals of one day. The heart-punctures sometimes resulted in the death of the bird at the drawing of the first sample. This accounts for the "single determinations" separately listed for the F_1 hybrids in Table I. An examination of the distribution of deaths from nearly 1,000 heart-punctures indicates that death more frequently resulted in (a) diseased birds, and (b) in birds carrying eggs nearly ready to lay or to ovulate. The prevalence of disease in these birds and the questionable value attaching to an unchecked determination warrants our exclusion (or separate presentation) of the values thus obtained.

Since our earlier work (6, 9) has shown that the blood sugar increases in the female at the ovulation period, and since the heart-puncture was found occasionally to cause the resorption of an ovum in the ovary or to cause it to pass into the body cavity, it was necessary to obtain samples in females with resting ovaries and also necessary to autopsy the birds within one to three days of sampling in order to make sure that the ovaries were really resting. And since diseased birds should ob-

viously be excluded, or be given separate consideration as is done here, it was necessary to kill the males also. It is of course only disease discoverable to us at autopsy that has been excluded, and probably a very few birds otherwise diseased are included in our groups of apparently healthy birds. Only non-ovulating sugar values enter into the data presented here. The sugar determinations were made by the Maclean micro-method. Further details of method, including precautions taken in the handling of the birds, have been described elsewhere (5, 9).

DATA

Since the actual amount of sugar in the blood is subject to some fluctuation, notwithstanding the several precautions taken by us, and because of the small but appreciable error in the method of determination itself, and finally because any individual may have been significantly even though not discoverably diseased, it is usually necessary in a study of the present kind to attach more significance to results obtained for a *group* than to even well-checked results for an individual. This is a most serious limitation if the end in view were a complete and definite account of the behavior of a character in inheritance. But a thorough analysis lies wholly outside the scope of our purpose, since besides various limitations of method and material no F_2 generation can be reared from these generic hybrids, and we must deal chiefly with the P_1 and F_1 generations. We do, however, attempt a small amount of individual treatment of the data obtained from the two F_1 series studied by us.

Table I gives the details of results obtained from the various groups concerned in the cross of *Turtur* with *Streptopelia*. It will be noted that for 10 pure *Turtur orientalis* which proved healthy at autopsy we find 188 mgms sugar per 100 cc of blood. That this very high sugar value is not in excess of what we may accept as normal for this species is further indicated by the fact

TABLE I

SUMMARY OF BLOOD SUGAR DETERMINATIONS ON TWO PARENT SPECIES
BELONGING TO DIFFERENT GENERA; ON THEIR MALE AND FEMALE
F₁ RECIPROCAL HYBRIDS; AND ON HYBRIDS BACK-CROSSED
TO ONE PARENT SPECIES

Description of birds		Sex	No. of birds in group	Average (or mean)	
				Body weight	Milligrams sugar per 100 cc. blood
Parent species	Turtur orientalis	♂ + ♀	10	221	188
	Streptopelia alba-risoria	♂ + ♀	10	171	149
	All duplicate sugar determinations				
One half T. orientalis (F₁)	Hybr. (♂ Turtur × ♀ Strep.)	♂	17	199	174
		♀	13	194	169
	Hybr. (♂ Strep. × ♀ Turtur)	♂	16	203	166
		♀	19	184	158
	Mean of 4 groups		......	*195*	*167*
	All single sugar determinations				
	Hybr. (♂ Turtur × ♀ Strep.)	♂	4	191	150
		♀	2	188	155
	Hybr. (♂ Strep. × ♀ Turtur)	♂	4	202	155
		♀	6	187	163
	Mean of 4 groups			*192*	*156*
	Healthy birds only and duplicate sugar determinations				
	Hybr. (♂ Turtur × ♀ Strep.)	♂	12	194	174
		♀	9	197	169
	Hybr. (♂ Strep. × ♀ Turtur)	♂	9	204	165
		♀	8	191	160
	Mean of 4 groups			*196*	*167*
Five eighths Turtur orientalis[3] (♀ 1/8 Turtur back-crossed with ♂ Turtur)		♂	1	199	160
		♀	6	203	174
One fourth Turtur orientalis[3] (F₁ back-crossed to ♀ Strep.)		♂	4	170	164
		♀	4	166	151
Mean of 2 groups				*168*	*158*
One thirty second or one sixty fourth Turtur[3] (back crosses to ♂ and ♀ Strep.)		♂	2	171	164
		♀	6	155	151
Mean[4] of 2 groups				*163*	*157*

[3] Healthy birds only.

[4] The "mean" signifies the unweighted average of the two (or four) average values.

25

that 12 additional birds thought to be healthy, but too valuable to kill (used in Figure 2), gave a value of 190. And the seven additional diseased birds examined gave an average of 198 mgms; two of this group gave extremely low and four gave very high values. It has been shown by still other work that the ring doves (*Streptopelia*) are properly measured by the 149 mgms obtained from the 10 healthy individuals listed in Table I. This value is sufficiently confirmed by figures obtained for the two following groups: First, the 12 apparently healthy birds (too valuable to kill) added to the 10 known healthy birds in Figure 2; these gave an average of 150.6. Second, three small fraternities (parent and grand-parents) shown in Figure 3 gave a like result; only *Streptopelia* is represented there. The fraternity of the male grand-parent (*St. risoria*) gave a value of 152, the female grand-parent (*St. alba-risoria-douraca*), 148, and the parent fraternity, 147. These 14 *Streptopelia* again average 149 mgms sugar per 100 cc of blood. In a cross of these species of *Turtur* and *Streptopelia* it is evident that we are making a cross of sugar values which rather closely correspond to 188 and 149 respectively.

Three readily understood divisions of the data obtained for the F_1 hybrids are given in Table I. In each division four groups represent the necessary separations for the sexes and for reciprocal crosses. The third or last of these principal divisions should supply the most reliable results. It will be noted, however, that the larger numbers of (duplicate) determinations of the first division gives a result practically identical with that shown in the third division, although the first division includes a number of diseased birds. But the second group—including smaller numbers, unchecked determinations and a high proportion of diseased birds—gives values for both blood sugar and for body size which do not check well with the more complete and more reliable data. It is, nevertheless, notable that all of the four groups of this defective division, as well as of the eight groups of

the two other divisions, *show a sugar value for the
hybrids which is intermediate to those of the two parent
species which entered the cross.* The mean value (167
mgms) obtained for the third (and first) division is an
almost exact intermediate of the values found for the two
parent species—being 21 points less than *Turtur* and 18
points greater than *Streptopelia.*

In a series of "five eighths" Turtur hybrids seven
birds gave an average[1] value of 172 mgms. Thus, this
group, which stands nearer pure *Turtur* in composition
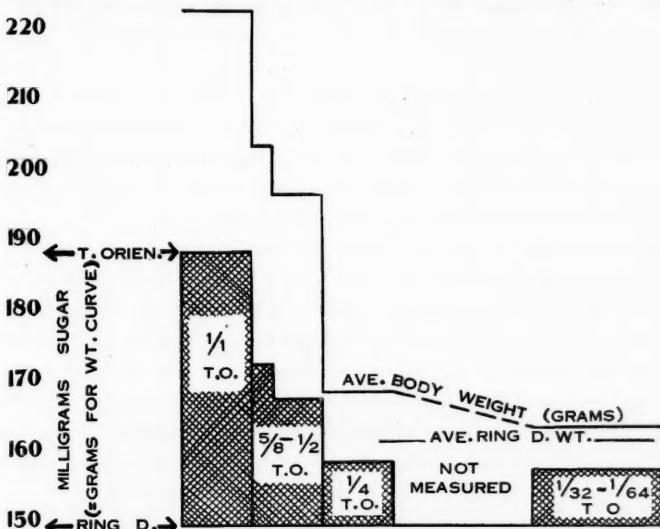
FIGURE 1

The rectangular blocks indicate the amounts by which the blood sugar
values of *T. orientalis* and its various hybrids (five eighths; one half; one
fourth; one thirty second) from crosses with the ring dove (*Streptopelia*)
exceed the sugar value of the ring dove parent (the base line).

The uppermost line records the mean body-weight of the identical birds
of the group whose mean blood sugar value is expressed in the rectangle
below. The very similar and repeated "fractionations" of the body-weight
and the blood sugar in the hybrids suggest that the same method of in-
heritance probably holds for both weight and sugar values.

[1] The *average* is probably better used here than the mean, particularly
since there is only one male, and since here only is the male indicated to
have a lower sugar value than the female. In the case of all other hybrid
groups the *mean* was used in the construction of Figure 1.

than do the F_1 hybrids, also more nearly approaches the sugar level of *Turtur* than do the latter hybrids.

The eight "one fourth" Turtur hybrids—arising from three different matings of F_1 hybrids (F_1 reciprocals both represented) back-crossed with *Streptopelia* females—again give a value (158 mgms) intermediate to that of their parents (F_1 and *Streptopelia*). The last class of these *Turtur* $\times$ *Streptopelia* hybrids—including eight birds which were only "one thirty second" or "one sixty fourth" Turtur—gave a mean sugar value of 157 mgms (average, 154) which is again an intermediate between the "one fourth" Turtur hybrid and the pure *Streptopelia*.

The above results are diagrammatically represented in Figure 1. The base line there represents the blood sugar level of the *Streptopelia* parents; and the shaded areas indicate the amount which this is surpassed, first by pure *Turtur* and later by the various hybrids of *Turtur* and *Streptopelia* in the order of their fractional or compositional relationship to *Turtur*. This diagram clearly indicates that the blood sugar value of the hybrids was "fractionated" or divided in the three (or four) graduated dilutions, or divisions, of the amount of *Turtur* represented in the offspring. If the reader wishes to visualize the total absolute sugar values of all groups, instead of their differences, he can do so by imagining the base line (149) carried downward at all points to zero.

A curve for the "body weight" of the several groups of birds which supply the blood sugar data is added to the uppermost section of the figure. This has been done to facilitate a comparison of the behavior of size and blood sugar in inheritance. The marginal figures which indicate "milligrams of blood sugar per 100 cc of blood" may be converted into "grams body weight" for the reading of this curve. In order to indicate the base of this curve, *i.e.*, the normal ring dove weight, we have not used the figure given in Table I for the 10 ring doves used there, but the value (161 grams) indicated by a much

larger previous series covering the season at which the majority of the hybrids of this study were weighed and killed.

Since one would like to know something of the individual sugar values and the range of variation which gave the mean intermediate value (167 mgms) for the F_1 hybrids of this cross, we have supplied these data in the
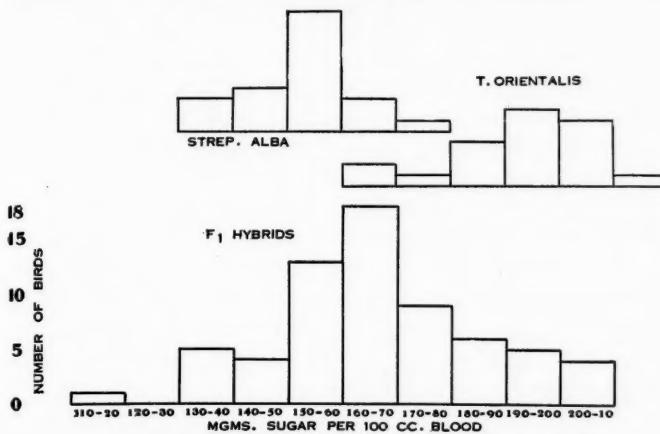
FIGURE 2

Polygons showing distribution of blood sugar values obtained from 65 F_1 *Turtur* × *Streptopelia* hybrids, and from 22 individuals of each of the two parent species (*Turtur orientalis* and *Streptopelia alba*).

form of a polygon in Figure 2. This figure makes it clear that the mode of this polygon corresponds with the mean value (167 mgms; average, 166) and that our mean and average values are not derived from the combination of numbers obtained from a bimodal curve. In other words, the values obtained are distributed as are the values from characters which in F_1 show "intermediate" behavior in heredity. In order to have larger numbers of F_1 for this polygon, all the birds—including some unhealthy ones— of the first division of Table I are here utilized. And in order to have larger numbers for the construction of similar polygons for the two parent species we have here added to the 10 healthy individuals listed in Table I, an-

other 12 individuals of each of these species which were all apparently healthy but too valuable to kill and verify this point.   The polygon of each parent species therefore includes 22 individuals.   The average blood sugar of the 22 *Streptopelia* represented is 151; of the 22 *Turtur,* 190.   It is then also clear that these polygons for the parent species show a distribution about a single mode, and that this modal point includes the average blood sugar value found in the group as a whole.

The results obtained from a second generic cross—a male *Spilopelia suratensis* mated to a female *Streptopelia* hybrid—are diagrammatically represented in Figure 3.   The blood sugar level of the *Spilopelia suratensis* is known only from the data obtained for the parent (♂ P870) used in this cross; but the value (170 mgms) obtained for this individual is the result of three well-checked determinations.   One individual of another species of this genus—*Spilopelia chinensis*—gave 173 mgms sugar.   The sugar value obtained for the female parent of the hybrids of the cross now being discussed requires special consideration.
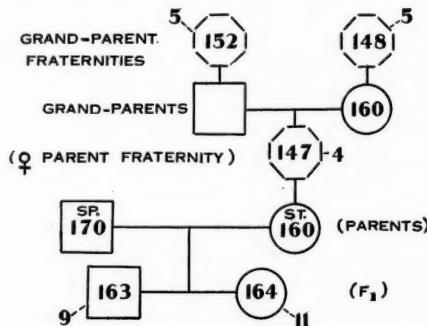

FIGURE 3

Family history, in terms of blood sugar values, of 20 (9 ♂ and 11 ♀) healthy generic F_1 hybrids and of their male (SP. 170) and female parents (ST. 160).   These hybrids show an average and a mean sugar value intermediate to the values of their parents and of the genera to which their parents belong.

The blood sugar value given in Figure 3 for the female

parent (P995) is 160 mgms. This is of course the value
obtained; but the autopsy revealed that this was not a
true non-ovulation value—an unsuspected ovulation into
the body cavity having occurred about two days before
the blood samples were drawn. Other work has shown
(6, 9) that the sugar value increases at ovulation and that
about 108 hours must then elapse before the blood sugar
again returns to normal. Under these conditions the
sugar value obtained was almost certainly too high. But
we have the following evidence concerning the sugar
value which she would be expected to transmit to her off-
spring: Sugar determinations made on four individuals
of her own fraternity gave an average of 147 mgms; her
mother gave a value of 160, but this also was probably ab-
normally high since five determinations on individuals
of her own family (fraternity and parent) gave a value
of 148 mgms; again, five members of her father's family
gave a value of 152 mgms. The average for the 14 birds
of her own fraternity and ancestry was therefore only
149 mgms sugar. This is exactly the value obtained by
us for ring doves in general and is the probable value
which female P995 would transmit to her offspring.

From the preceding data and discussion it may be con-
cluded that in this second generic cross a sugar value of
about 149 was crossed with another of approximately
170. The two groups of hybrids obtained—male and
female—are both shown in Figure 3 to have had blood
sugar values intermediate to those borne by their two
parents. Apparently these values in the offspring are
nearer to that of the *Spilopelia* parent; they would be ex-
actly intermediate if the true blood sugar value of *Spilo-
pelia* should prove to be about 178. Whitman (16) has
shown that *Spilopelia* has a closer phylogenetic relation-
ship to *Turtur* than to *Streptopelia* and this would lead
to an expectation of a higher value than 170 in *Spilopelia*
rather than of a lower value. And we have already noted
that a single individual of *Spil. chinensis* gave a value of
173. Unquestionably both groups (males and females)
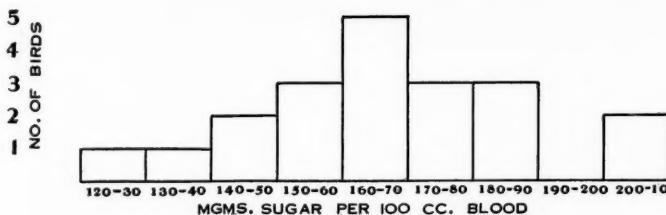
FIGURE 4

Polygon showing distribution of blood sugar values obtained from 20 healthy F_1 *Spilopelia* $\times$ *Streptopelia* hybrids.

of these hybrids have values distinctly above the known sugar values of *Streptopelia*; and they have a smaller value than that found for their *Spilopelia* parent.

It is of interest to note that the F_1 hybrids described above have a lower sugar level than have the hybrids from the comparable ♂ *Turtur* $\times$ ♀ *Streptopelia* cross shown in Table I—the males there giving 174 against 163 here; the females 169 there against 164 here. The *Turtur* parents of the earlier cross had a sugar value of 188, while the *Spilopelia* parent of the present cross apparently had a sugar value of only 170. Figure 4 supplies the evidence that the average values obtained here do not result from a combination of values from a bimodal curve. The polygon of distribution of the F_1 hybrids is again such as to indicate that the character crossed behaves as do intermediate or blending characters.

We have elsewhere (10) given full consideration to the question whether there is a sex difference in the blood sugar level in males and females of pure species. On the question of such a sex difference in hybrids we possess only the data presented above. The sex differences found in hybrids from the *Turtur* $\times$ *Streptopelia* cross are perhaps sufficient, if they stood alone, to suggest a higher value in the male hybrids. But the data for the *Spilopelia* $\times$ *Streptopelia* hybrids do not support this view and the point must be regarded as quite uncertain. Do the reciprocal hybrids have equivalent sugar values? On

this matter also our data are all given here and the results from the single group thus tested (*Turtur* $\times$ *Streptopelia*) seem to indicate a higher sugar value in the hybrids when *Turtur* is used as the male parent. It is possible, however, that if duplicate determinations had been obtained from the 16 birds with unchecked determinations (second division of Table I) their checked values would have been quite close to those tentatively tabulated for them, and that significant differences for both reciprocal crosses and for sex in hybrids would thus be largely eliminated. Conclusions on these points can not now be drawn; but these possible differences are noted as the reason for using *mean* values instead of averages in dealing with the data of Table I. Fortunately, the mean and the average are almost identical for most of the groups represented in that table.

DISCUSSION

The data of the present paper clearly indicate: (a) That in the two generic crosses made by us the blood sugar of the parent genera stood at distinctly different levels; (b) that the F_1 hybrids from both of these crosses had values intermediate to those of the parent genera; (c) that this intermediate value in the one tested case again divided or "fractionated" in a back-cross with one of the parent species; (d) and that in still other fractional parts of parental composition (five-eighths, one-thirty-second) there is found some evidence that the sugar level is either further divisible or that it still shows the presence and hereditary effect of both parental genera. The data are not of such nature as to test the question as to whether segregation does or does not occur and are otherwise obviously limited in amount and in their application. But the character here studied is a less superficial one than are the visible characters whose hereditary behavior have become well known, and the possibility of an influence of this more deeply-seated character upon those visible and more superficial characters is a point of interest.

TABLE II

Approximate and Comparative Amounts of Reducing Substances in the
Blood of Various Animals and in Seven Genera of Pigeons

Animal	No. of		Reducing power of blood (milligrams sugar per 100 cc blood)	Observer
	Animals	Determinations		
Man	......	61	84	Strouse
Horse	......	2	70	Abderhalden
Sheep	......	2	70	Abderhalden
Cat	......	16	70	Scott
Dog	......	7	75	Scott and Hastings
Rabbit	......	25	118	Scott and Ford
Duck	......	14	150	Kausch
Goose	......	6	150	Kausch
Fowl	......	9	200	Saito and Katayama
Tortoise	......	4	90	Bierry and Giaja
Frog	......	26	40	Scott and Kleitman
Dog-fish	......	4	65	Scott
Chimaera	......	......	28	Lang and Macleod
Cancer	......	......	40	Lang and Macleod
Octopus	......	......	32	Bierry and Giaja

Various Genera of Pigeons

Animal	Animals	Determinations	Reducing power	Observer
Zenaidura carolinensis	2	5	257	Unpublished
Turtur orientalis	22	43	190	Riddle and Honeywell
Leucosarcia picata	1	5	190	Unpublished
Common pigeon (Columba)	11	22	185	Scott and Honeywell
Common pigeon (Columba)	37	103	181	Unpublished
Spilopelia chinensis	1	3	173	Unpublished
Spilopelia suratensis	1	3	170	Riddle and Honeywell
Stigmatopelia senegalensis	9	15	168	Unpublished
Streptopelia risoria Streptopelia alba	22	36	151	Unpublished

The curve for "body weight" which accompanies the data for blood sugar in Figure 2 is so similar to the curve for the latter as to permit the inference that the mechanism for the inheritance of the one applies also to the other. It must be pointed out that among doves or other animals there is no direct relation between body weight and blood sugar level such as would naturally result in the parallelism seen in Figure 2. The species with the highest blood sugar shown in Table II (*Zenai-*

dura) is with one exception the smallest species represented in the table; the smallest species (*Stigmatopelia*) has, with two exceptions, the lowest sugar, etc. Nor within the limits of a dove species is there any definite relation between the body weight and the blood sugar. The multiple factor hypothesis is the rather widely accepted interpretation of the more usual behavior of "body size" in inheritance. This interpretation is supported in most studies of this subject by one or another amount of observable and measurable segregation. Whether segregation of blood sugar values actually occurs is, as stated above, a question not tested by our data; we may add, however, that we do not therefore assume that it does occur. On this point our own view is that it is fairly clear that the most fundamental characteristics or properties of living matter—irritability, respiration, contractility, growth, etc.—obviously do not segregate, and that a multitude of visible characters certainly do segregate. Whether the normal hereditary level of blood sugar goes with the one group or with the other is quite unknown. The relatively large rôle of three to several glands of internal secretion, and of a part of the nervous system, in the normal maintenance and regulation of the blood sugar level would seem, however, to afford a logical basis for the assumption that the blood sugar level may have a "multiple factor" basis and that some or many of these factors may be segregable.

A few available observations bear in some degree on the point just mentioned. Pike and Scott (8) have called attention to the fact that a mechanism for maintaining the constancy of the sugar of the blood is one of a number of internal adjustments which the progressively higher animals have had to develop in greater and greater perfection. And Scott (14) has further suggested that in higher animals a delicate secondary mechanism has been developed and passed on through inheritance which permits a rapid variation of the blood sugar about the species norm as an emergency means of

securing a prompt release of energy. All of these considerations emphasize the fact that the sugar of the blood is both a regulated and a regulating mechanism; and that its changes—whether of permanent or temporary nature—are of much greater significance to cells and tissues generally than are changes in the ordinary visible characters with which our studies of inheritance commonly deal.

It was noted at the beginning of this paper that studies on the inheritance of characters comparable with blood sugar values have not been made.[2] In this connection it should perhaps be remarked that the behavior of starchy and sugary characters—which are really alternative forms of the same thing—have received much attention in plant genetics. In maize crosses, for example, the chief facts for starchy and sugary endosperm have been reported by Correns (1), East and Hayes (2) and others. A paper by Pearl and Bartlett (7) supplies quantitative determinations of starch, various sugars, moisture, etc., in P_1, F_1 and F_2, from a one-direction cross made with two varieties of corn. In all the above work with maize a segregation of these sugary and starchy characters is shown to occur in the F_2. The F_1 endosperm of corn is of course not wholly comparable with the F_1 of the corn plant nor of the F_1 animal organism. The results of none of these studies seem really comparable with a character such as the blood sugar which, in the animal body, is under continuous regulation and whose temporary or permanent fluctuation in the circulating fluid calls forth other equilibrium changes in both the blood and in all tissues supplied with blood. That such equilibrium changes or adjustments on the part of blood and tissues accompany the permanently high or low sugar level follows necessarily from the general physico-chemical rela-

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[2] Since 1920 our colleague, Dr. J. Arthur Harris, has been conducting extensive investigations on the physico-chemical properties of the tissue fluids of Egyptian and Upland cotton, and of their hybrids, as grown under irrigation agriculture at the Cooperative Testing Station, U. S. Department of Agriculture, at Sacaton, Arizona.

tions involved. But a special consideration of some aspects of these adjustments to altered concentrations of the blood sugar has already been supplied by Epstein (3) and by Woodyatt (13, 17).

The blood change found and described by Gerould (4) in blue-green caterpillars is a quite different matter from the subject under consideration but may be briefly mentioned here. In this case the mutational character involved the destruction or decolorization of xanthophyll in its passage through the intestinal epithelium. As a result of this destruction the caterpillar's blood was free of the pigment, and all the various parts of the animal's body which were normally pigmented with xanthophyll through the blood supply were left without this pigment in all stages of the life-history of the insect.

The foregoing results lead to the suggestion that the intermediate blending or divisible nature of such characters as the blood sugar level in the F_1, and a second divisibility in the F_1 back-crossed to a parent genus, affords a possible explanation of some observed irregularities in the behavior of visible characters in wide crosses as this has been observed particularly by Whitman (15). Wide differences of such a character as blood sugar may usually be expected only in forms rather widely separated phylogenetically. That this is true in the case of blood sugar itself is indicated by the data assembled in Table II. From crosses of different genera of pigeons—where fertility extends even to different families—Whitman observed a number of phenomena which it is of interest to review in the light of the facts given above.

The final chapter of the second volume of Whitman's studies is devoted to a consideration of ''The divisibility of characters.'' It was there shown that the F_1 obtained from crosses of wild species and genera of pigeons exhibit (sex limited characters partially or largely excepted) a blending of the parental characters. Moreover, the back-crosses of these F_1 hybrids again show a second

or even a third "division" of the parental characters. Involved in these cases were such characters as general body coloration, the "neck-mark" as a whole, and both the coloration and bifid condition of the individual feathers within this neck-mark. We may note here that the analysis of the divisibility of the blood sugar level observed by us in the *Turtur* × *Streptopelia* cross quite closely parallels the behavior of the visible characters of these same hybrids as studied by Whitman. Again, our second case (*Spilopelia* × *Streptopelia*) involves the identical cross in which Whitman has described (15; see plate 34) a second "division" of the three above-mentioned characters of the neck-mark. Hereditary behavior of the above kind Whitman called "pure or direct inheritance."

A second type of irregularity of hereditary behavior encountered in these crosses of different genera by Whitman is concerned with the impurity or incomplete dominance or recessiveness of characters, notably of color characters; this being sometimes found even in cases of sex-limited color inheritance. For example,— in the very same birds used as principal material for the present study—the white color of *Streptopelia alba* never emerges even approximately pure white from crosses with *Turtur,* but shows various areas of partial pigmentation. These cases could also of course be "described" in terms of multiple factors, modifying factors, contamination, etc. The possibility that unequal numbers of chromosomes are contributed by these genera might also be considered. The results of this study, however, raise the question: Are the new and different levels of blood sugar—and of other similar though as yet unstudied elements introduced into the hybrid by wide crosses—perhaps these modifying or contaminating factors? The new and different blood sugar level of the hybrid certainly provides a somewhat different internal environment in which the characters proceeding from the parent forms must develop. Is no mark left upon the

multitude of visible characters which must undergo the whole of their development under this new, persistent and intimate environment?

Still a third large group of cases of aberrant developmental or hereditary behavior is encountered among the hybrids proceeding from many wide crosses. The exhibition of fertility in all degrees and the fixed or limited life-terms of offspring in some of these crosses constitute one group of such characters; and the greatly increased frequency of reproductive abnormalities and of absent gonads in these offspring supplies us with another.

In none of these cases cited above is it necessary to suppose that an altered sugar level alone, nor that an alteration of any other single one of the several invisible characters which probably share the blood sugar mode of inheritance, is the sole cause of the aberrant behavior. The important point would seem to be that a new internal (blood) environment is established in such hybrids and that the parental characters show a developmental response to this new environment. We have here been able to show that one important element of such a new blood environment does occur in some of these hybrids; and we have noted that some of the characters of these same hybrids show atypical or unexpected hereditary behavior. Elsewhere we have shown (10) that the normal sugar level extends throughout the life cycle in the pigeon, and is already present in the embryo.

At the beginning of this paper it was noted that from some reported crosses of species or of genera there result, apparently, no notable departures from expected hereditary behavior. We are now able to offer a possible explanation for the issuance of normal hereditary behavior from certain wide crosses and abnormal or unexpected behavior from certain others. For, in some such crosses the blood sugar level—and other similar regulating characteristics of the blood or internal environment—may not be notably different in the forms crossed;

while other crosses do involve wide differences in these characteristics of the internal environment. In these latter cases the continued "fractionation" of such characteristics of the internal environment assists in the continued blending or fractionation of characters in the hybrids of the various grades. It is thus made evident that in the case of some wide crosses an agency for the modification of developing characteristics may come into action which is necessarily absent from crosses of closely related races, varieties and species.

On the basis of the facts obtained from the present study of the blood sugar of dove hybrids it is reasonable to assume that there exist other as yet unstudied characteristics of the blood—or internal environment—which behave in inheritance in the same way as does the blood sugar level. Some of these characteristics, states or processes may affect developmental processes more readily than do others. We venture the suggestion that some or many of the visible characteristics of two parent species which have developed in, and are presumably perfectly adapted to, a blood environment at one level may exhibit a response of one or another grade when forced to develop in a different blood environment in the soma of the hybrid. If this is true we are in a more favorable position to interpret some of the hereditary and developmental phenomena presented in hybrids from wide crosses.

Summary

The genera *Turtur* and *Streptopelia* were observed to have quite different amounts of sugar in the blood—respectively 188 and 149 mgms sugar per 100 cc of blood —and a study was made of the amount of blood sugar in various grades of hybrids of these two genera. A study of F_1 hybrids of a *Spilopelia* $\times$ *Streptopelia* cross was also made.

The F_1 hybrids obtained from both crosses had blood sugar values intermediate to those of the parent genera.

The offspring of F_1 hybrids of *Turtur* $\times$ *Streptopelia* back-crossed to *Streptopelia* again gave sugar values intermediate to those of the F_1 and *Streptopelia*. Still other grades of these hybrids obtained from back-crosses apparently also show a further blending or "fractionation" of this character.

The blood sugar level is apparently inherited in the same way as is body size in these same animals.

The behavior in inheritance of a character comparable with the normal blood sugar level has hitherto not been studied. The hybrids examined had a blood sugar value different from the values of the parent genera; this different value is maintained throughout the life cycle of the hybrid; and this different sugar value requires equilibrium adjustments on the part of other blood and tissue components of the hybrid in all or nearly all stages of the development, growth and life of the hybrid.

It is suggested that certain irregular and other little known hereditary or developmental phenomena often met with in hybrids proceeding from wide crosses are the result of the circumstance that in such hybrids the parental characters are forced to undergo the whole of their development in blood which constitutes a new, intimate and continuously acting internal environment.

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EXPERIMENTAL ACCURACY IN FRUIT BREEDING

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PRIOR to 1900, pomological crops were bred and improved like other agricultural crops in a more or less empirical manner. The awakened interest in a more thorough investigation of pomological plants subsequent to 1900 is reflected in the statement made by U. P. Hedrick in regard to grape breeding. ''The ultimate aim in this work is, of course, the production of improved horticultural varieties. Through the early days, when breeding laws and methods were less understood than now, there was a tendency to make this the immediate as well as the ultimate aim. The fact that the first twenty years of grape breeding produced but one variety worthy a name served to confirm the conviction that this goal would be reached quicker by forgetting it for the time being and bending every effort to the discovery of how grape characters are transmitted.''

The importance to agriculture of accurate fruit breeding experiments may be illustrated by a single example. Among peach varieties there are those which are characterized by a considerable amount of anthocyanine pigment in the twigs, while others lack this coloring matter and hence have green twigs. The colored varieties absorb considerable heat and during the warm periods in spring the buds begin growth and hence are liable to injury during subsequent cold periods. Since the green twigged sorts are not subject to this type of injury they are more desirable in localities where cold periods occur during the year. The green twigged types have been found wild in the southern mountains and have a poor quality of fruit, while the colored forms include most of the commercial varieties. The recombination of such de-

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sirable commercial characters will be of great signifi-
cance to horticulture. The employment of the proper
breeding experiments is essential in the creation of such
varieties. For example, if the green twigged character
is recessive it would be necessary to raise the second gen-
eration and to control the pollination of the F_1 trees from
which the second generation was obtained. Since this
problem is the subject of investigation of another experi-
ment station, I have referred to it as an illustration only
in a very general way.

The analysis of the inheritance of characters in such
large perennial plants requires a considerable period of
time as well as a large expenditure of funds. For this
reason genetic investigations with pomological plants
have not made the same advances as such investigations
in shorter life cycled plants. With the exception of such
phenomena as bud mutations, senility and extended stud-
ies of hybrid vigor, fruit plants do not offer the most
favorable material for genetic investigations. Such in-
vestigations have more of a utilitarian object than a
scientific one in view. Due to this fact many of the tests
performed in a genetic analysis may have to be omitted,
but a classification of the particular phenotypes which
are grown might be of considerable value in further
breeding experiments. Though this is true, it should not
alter the accuracy employed, but on the contrary, one
would naturally suppose that, in breeding forms so costly
in time and resources, the most exacting technique would
be one of the first essentials.

The greatest advances in any of the commercial fields
have resulted from the utilization of scientific discov-
eries. This will be true in pomology and any agricultural
field as well as in chemistry or engineering. Disregard-
ing the question of methods to employ in commercial
breeding the criticisms in this paper are justified because
the articles reviewed purport to give a genetic classifica-
tion and interpretation of certain fruit breeding experi-
ments. In looking over the breeding literature along

pomological lines, one is usually impressed by the inaccuracies of an experimental nature and the misinterpretations which are employed.

One of the first prerequisites for the establishment of accurate genetic facts or for the isolation of desired recombinations is the use of sufficient numbers. The employment of a number of crosses with a few individuals each is one of the commonest failings of fruit breeders. If any genetic experiment requires large numbers an investigation of horticultural plants certainly would because of the heterozygosity of many of the varieties.

Babcock and Lloyd (1917) have pointed out that the restriction of the use of scientific terms to a given set of conditions is necessary for mutual understanding. Likewise the discussion and interpretation of genetic data according to logical scientific reasoning is equally essential to mutual understanding. In the remainder of this article I will try to point out a few of the inconsistencies of this nature which have occurred in fruit breeding literature within the last few years.

Hedrick and Anthony (1915) crossed grapes having abortive pollen and normal pistils with those having normal pollen and rudimentary pistils. From this cross they obtained 56 hermaphrodites and 51 with rudimentary pistils. When perfect hermaphrodites were selfed only perfect hermaphrodites were obtained. Vines with abortive pollen were referred to as hermaphroditic females and forms with rudimentary pistils as pure males. The discussion of the inheritance of these forms was referred to as a case of sex inheritance. They state that

this condition might be covered by the assumption that the hermaphrodite is a female in which the addition of a single dose of maleness has caused the production of male organs, the ovules keeping the composition ♀ ♀ and the pollen becoming ♂ ♀.

Hermaphrodite × hermaphrodite = ♀ ♀ × ♂ ♀ = 2 ♂ ♀ + ♀ ♀.

Since we have no pure females, we must assume that some condition prevents the formation of individuals with the composition ♀ ♀; therefore, the above cross gives only hermaphrodites. Of course, if we do not attempt to assume the method of origin of the hermaphrodite, the case may be covered by considering the hermaphrodites pure for this character, while the males would be heterozygous.

$$♀ ♀ × ♀ ♀ = ♀ ♀$$
$$♀ ♀ × ♀ ♂ = ♀ ♀ + ♀ ♂.$$

The misconceptions of this explanation are evident to any one who is familiar with modern cytology. If we take their statements literally they assume a diploid condition instead of the haploid condition for the ovules and pollen. If one interprets their statements to mean that the ovules of hermaphroditic vines are all of one kind, let us say the X type, and the pollen of the same vine is of two types as X or Y, then we must devise some mechanism by which this could take place. Since their explanation is entirely inconsistent with present knowledge we would have to reconstruct a hypothesis based upon some of the known facts of sex inheritance in plants if their observations are to be included in this category. Their data, however, are not sufficient to justify anything except a purely speculative theory, and it will be pointed out in the following paragraph that the case can be explained without involving sex inheritance.

White (1914) has demonstrated that petalody and pistillody of the anthers of tobacco flowers are inherited characters. Since factors are known which cause teratological structures to develop on anthers it would be logical to assume that there might be factors which cause the suppression of pollen or pistils in grape vines. We could assume a recessive inhibiting factor (s) for pollen development and a recessive inhibiting factor (p) for pistil development. If the vine with defective pollen was represented by the formula Ppss and the vine with rudimentary pistils by ppSS, then the progeny from an immediate cross between these vines would be of two kinds, PpSs or perfect hermaphrodites and ppSs or vines with rudimentary pistils and these two types would occur in equal proportions. The correct explanation of the inheritance of defective sex organs in grapes will, of course, depend upon further breeding data. It is sufficient to point out that Hedrick and Anthony are not justified in their assumption of sex inheritance in grapes without further breeding tests.

The statement by Connors (1919) in regard to the in-

heritance of flesh color in peaches is even more inconsistent. "Since most of our peach varieties are chance seedlings, we do not know with any certainty what these varieties carry as recessives in flesh color. An example of this is the variety Elberta. This variety is known to be a seedling of Chinese Cling. If Chinese Cling were pure white and were crossed with a yellow variety then all the seedlings in the F_1 generation should be white, unless color of flesh is a sex-linked character." Peaches are a hermaphroditic species and hence sex-linkage is not possible according to the way this term is used by geneticists.

But the misuse of genetic terminology is not the greatest sin committed by fruit breeders. The accompanying table taken from Connors (1919) lists inconsistent reciprocal crosses without stating whether the same trees were used for these reciprocal crosses. This criticism is justified from the looseness in which other genetic terminology has been used in the same article. Reciprocal may have been used simply in the sense that the same varieties were employed. In order to have a reciprocal cross when working with fruit varieties, identical trees must be used in both crosses unless it is accurately known that another tree of the same variety was propagated from a second cutting of the bud wood from which the first tree came. In this connection it is sufficient to point out that in many orchards the trees have not been obtained from the same nursery. The present pomological varieties include trees of different origin and hence of possible different genetic constitutions. An inspection of Table III of his article indicates at once that there is something wrong with the reciprocal crosses. The fact that the two crosses, one involving Early Crawford $\times$ Belle and the other Early Crawford $\times$ Elberta, both gave inconsistent reciprocal results would throw doubt upon the Early Crawford parent which was used in these reciprocal crosses. A statement as to the identity of the parents would have been of great aid in the interpretation of the results.

Furthermore, the table shows that the results of two distinct crosses are added together for a total. This practice has been followed in a second publication dealing with the same fruit (Connors 1921). Such a procedure would have significance provided that it was previously demonstrated that Belle and Elberta carried identical genes for size of flower, but this was not done.

TABLE III[1]

	Large		Medium		Small		
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	Total
Belle × Early Crawford	1	0.8	76	63.3	43	35.8	120
Elberta × Early Crawford	11	14.1	46	59.0	21	26.9	78
Totals	12	6.1	122	61.6	64	32.3	198
Early Crawford × Belle	......	......	21	80.8	5	19.2	26
Early Crawford × Elberta	......	......	21	50.0	21	50.0	42
Totals	......	......	42	61.8	26	38.2	68

In another publication Connors (1921) described the seedlings grown from self-fertilized seeds of trees having reniform glands on the petioles and margins of the leaves. All of these varieties produced seedlings having leaves with reniform glands. Several of the varieties with reniform glands were crossed. Most of the crosses gave seedlings, all of which had reniform glands. One cross, however, Slappey × Arp, gave 23 seedlings with reniform glands and 6 with globose glands. This result is explained by the following statement. "It is possible that accidental mixture has taken place in this instance either at the time of harvesting the seeds from which the trees came, or in planting the trees in the orchard, because the six with globose glands resemble closely the seedlings from Slappey × Early Crawford." The publication of all the data of any experiment whether it con-

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[1] Large, medium, small refer to size of peach flowers. Early Crawford had small flowers while Belle and Elberta had medium sized flowers.

forms to expectation or not is highly desirable and small errors do occur occasionally in experimental work. The occurrence of such results as given by the cross Slappey × Arp could easily be due to the interaction of factors or to some similar genetic reaction. Nevertheless, the sentence would seem to indicate a lack of accurate control of experimental material, a very common error in so-called commercial experiments.

Beach and Maney (1912) have reported upon a cross between the western sandcherry (*Prunus besseyi*) and the Wyant plum (*Prunus americana*). "Number of serrations.—As a result of numerous measurements serrations to the inch averaged 9.7 on the sandcherry and 12.6 on Wyant. The classification follows. The results are inconclusive."

Group	Number	Per cent.
1	14	11.2
1a	29	23.2
2a	37	29.6
2	45	36.0
Total population	125	

It followed from previous explanation that the results tabulated were for the second generation and that the group classification had the following significance.

1 Individuals most closely approximating the type of the mother P_1.
1a Intermediates which most favor the mother type P_1.
2a Intermediates favoring the male parent P_1.
2 Individuals most closely approximating the type of male parent P_1.

Since the above quotation includes all the data which the authors present on the inheritance of number of serrations, one has no way to judge as to how clearly the different groups were separated from one another or in what manner the data were treated. Since the authors tabulated intermediate types and since number of serrations would probably be a quantitative character, the data should have been treated as a frequency distribution rather than an arbitrary classification into four different groups.

It is needless for the purpose of the present discussion to multiply examples of errors which have occurred in publications of several fruit breeders within the last few years. Similar flaws could be pointed out in some other types of pomological investigations of recent date. It would likewise be more useless to attempt to revise this literature since the data in most cases have not been presented in a form which can be interpreted. In most articles where the data are presented in a form which can be checked easily, the conclusions are stated equally well.

One may conclude by saying that the analysis of the hereditary constitution and the improvement of fruit plants offer a unique field for the trained investigator as well as being of great importance to agriculture. It seems almost a tragedy, however, to expend a great amount of time and funds for inaccurate investigation when accurate results could be secured equally as well.

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YELLOW FEVER AND FISHES

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CONSIDERABLE interest has developed in Ecuador and Peru by utilizing fishes to control yellow fever. Various fevers are communicated from person to person by the bite of various mosquitoes. No mosquitoes, no fever.

Any means that will suppress mosquitoes will suppress fevers. There are three ways to eradicate mosquitoes. Abolish the breeding places of mosquitoes. This is easier said than done. A swamp may be drained, but a tomato can or two left exposed to rain is sufficient to interest a whole neighborhood. It has been reported somewhere that a poverty-stricken college complained to a proverbially rich factory owner that his pond adjoining the college grounds was a mosquito nuisance. He offered the college a dollar for every mosquito larva caught in his pond. The students turned out in mass to catch mosquito larvae and earn dollars. But they did not earn a dollar because it was discovered that an old posthole on the college campus was providing the mosquitoes. Considerable energy has been spent draining swamps to get rid of the breeding places of mosquitoes.

The suppression of mosquitoes by oiling the surface of all standing water was the classic means employed in Panama.

It has been known for years that some fishes make away with some mosquito larvae. Probably most young fishes eat mosquito larvae. Some fishes are more effective mosquito larva eaters than others. It is current belief that the "millions" of Barbadoes, *Acanthophacelus reticulatus* (also native in Trinidad and along the lowlands of the Guianas) have kept yellow fever out of Trinidad.

In North America Gambusia plays the principal rôle

as a mosquito eradicator, and extensive plans are carried out by S. F. Hildebrand of the Bureau of Fisheries to distribute Gambusias to places where they are most needed and where they will do the most good.

Within the last few years, experiments were made near the coast of Ecuador and Peru to find mosquito eaters among the native fishes.

To serve as an ideal yellow fever mosquito larva eater, the fish must not only eat larvae, but it also must be able to live under the conditions in which yellow fever mosquitoes breed. Yellow fever mosquitoes live about and in the houses of man. They breed in the various water jars, cans and barrels in which drinking water is stored.

The fishes, therefore, must thrive in standing water. "Millions" were imported for the larger containers in Guayaquil and the following three native fishes, named in the order of their importance, were found to best serve the purpose of removing mosquitoes from houses.

1. The most important is "Life" (Fig. 1) (*Pygidium*
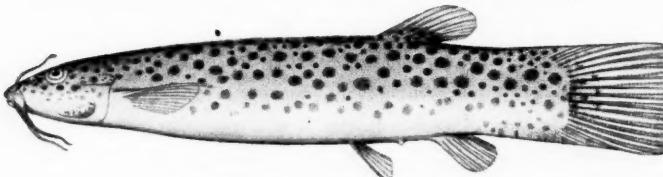
Fig. 1. "Life," Pygidium piurae E.

piurae E.), a small catfish belonging to a South American family of about 100 species. The genus Pygidium embraces over half, (sixty-five) of the known species. The different species differ but little in habit and appearance and probably all of them will serve as mosquito larvae eaters. They are found in all of the mountains of South America from the edge of Panama, at least to Puerto Montt in Chili. They have been caught from sea level all the way to 12,000 feet and more. They are hardy mountaineers lying at the bottom of torrents in daytime, coming to the surface at night to feed.

The map (Fig. 2) shows the general distribution of members of the genus Pygidium. I found a few about

SOUTH AMERICA
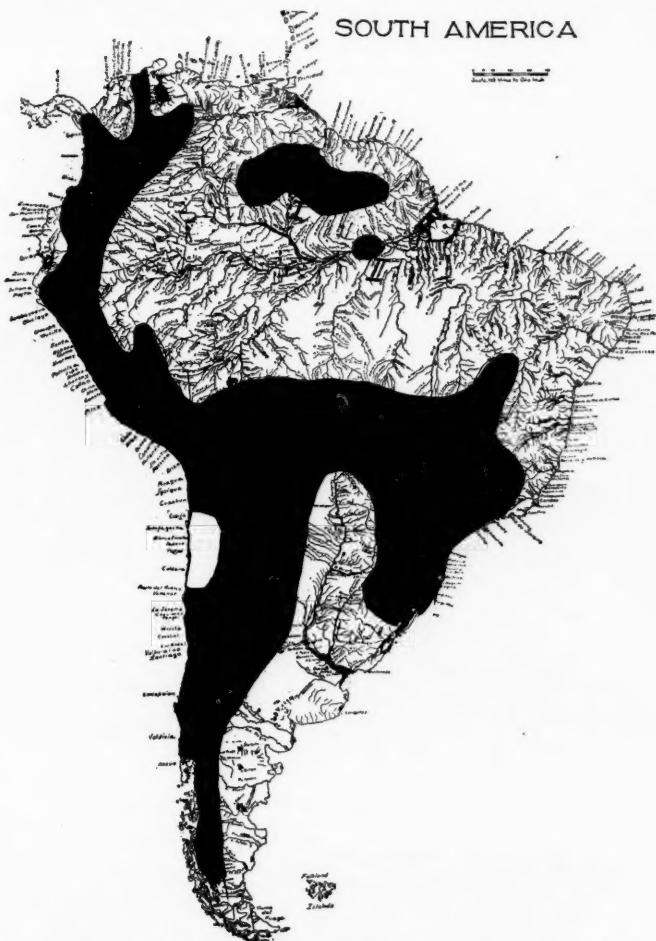
FIG. 2. Distribution of the genus Pygidium. They are most abundant in the cooler mountain streams. A very small species is found at the bottom, even burrowing in the hot Amazon Valley.

the cataracts in British Guiana and have put a hypothetical island, I, covering the highlands of the Guianas in the Pygidium-free lowlands. Similarly, a minute species has been recorded from II in the Amazon and it is possible that they are found throughout the Amazon Valley.

Without doubt their most extensive habitat is along

the slopes of the Andes and in southeastern Brazil. Actually no Pygidium has been recorded south of Puerto Montt where they are still found in great abundance. South of that point members of a closely related genus (Hatcheria) have been recorded.

Eight hundred thousand of "lifes," distributed to all of the water jars in the infected regions by Dr. H. Hanson, suppressed the last yellow fever epidemic in Peru.

The distribution map is a modification of one prepared for my monograph of this family. "The Pygidiidae" (Mem. Carnegie Museum VII, 1918, pp. 259–397, plates XXXVI–LVI).
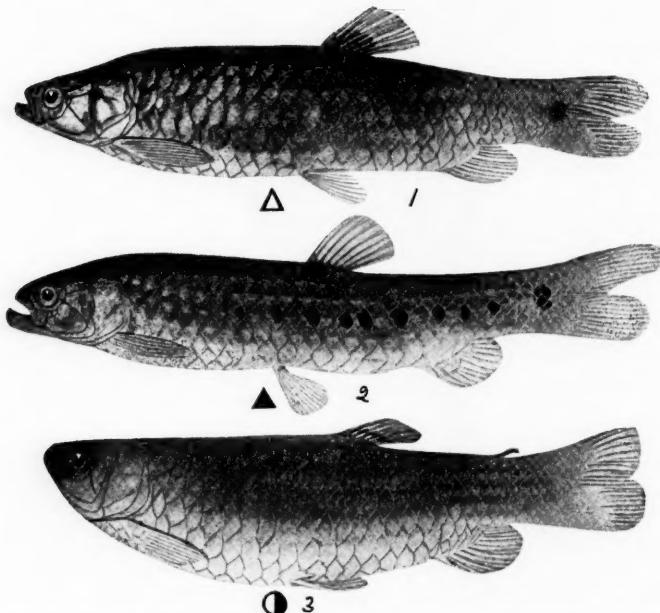
Fig. 3. The "Huaija," *Lebiasina bimaculata*, its nearest relative *L. multi-maculata* and a more remote relative *Piabucina panamensis*.

2. Liza de agua dulce of Lima and Huaija of Guayaquil are different names for the same fish—*Lebiasina bimaculata* (Fig. 3). This is a very active fish. It was independently discovered to be a larva eater by Dr. W. Pareja in Ecuador and by H. Hanson in Peru.
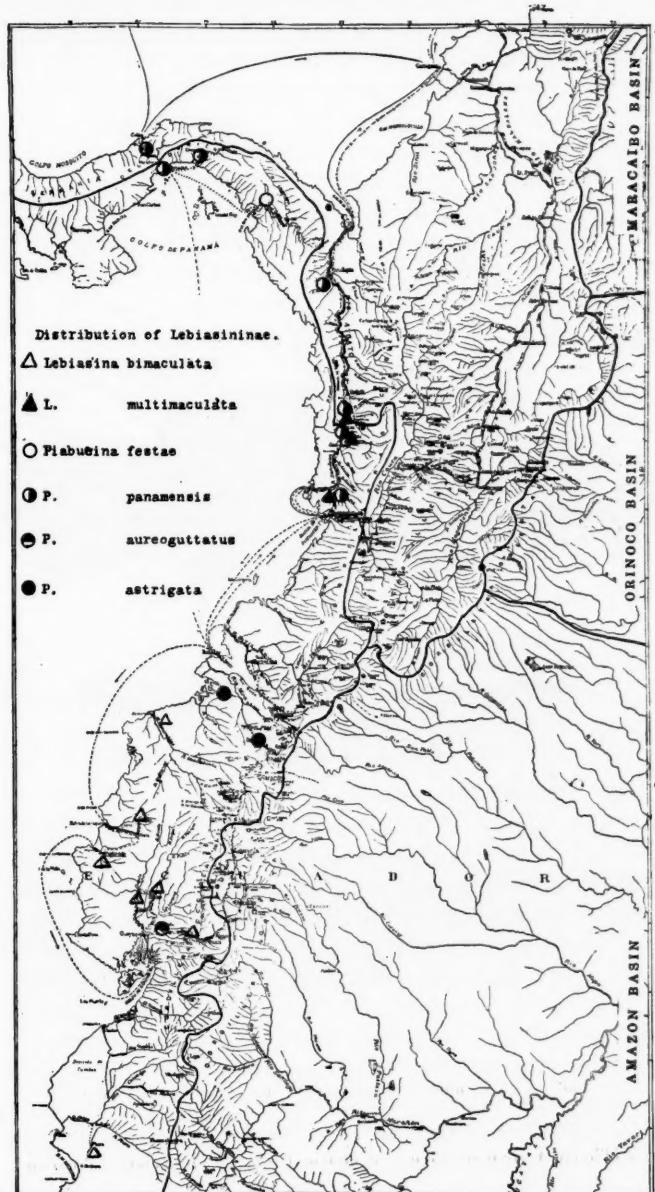
FIG. 4. The recorded localities in Ecuador and Colombia of the Huaicha and its relatives. The Huaicha itself extends south at least to central Peru.

I have taken it from sea level to over 7,000 feet, extending over more than the entire vertical range of yellow fever. Horizontally it occurs in all favorable places on the Pacific side between central Peru and central Ecuador. Related species are found between Guayaquil and Panama and species of a related genus are found on the Atlantic slope. In the restaurants at Lima this species is used as an ornamental aquarium fish. Its drawback is its tendency to jump out of the containers.

3. The Chalacos. This is a fish reaching over a foot in length and is found in tide pools and near the mouths of

Fig. 5. The ''Chalacos'' of the coast.

rivers from north Central Peru to California (Fig. 5). It has a ''double'' along the West Indian-Atlantic shores, and naturalists have veered from the opinion that the fishes on the two sides of Panama are identical to the notion that they are different. Certainly they are divergent forms of the same thing and the young of both sides ought to be available in mosquito work. Small specimens are said to do the work well, but do not survive the conditions as well as Lebiasina or Pygidium.

I have caught Pygidium near the coast in Northern Peru. As far as known in Ecuador, they only occur at considerable elevations. In Guayaquil Huaijas and Chalacos are so cheap that it may not pay to use Pygidium.

SYMBIOSIS IN ANIMALS AND PLANTS[1]

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INTRODUCTION

THE subject of symbiosis has been chosen for this address because of its broad biological interest, an interest that appeals equally to the physiologist, pathologist and parasitologist. It is, moreover, a subject upon which much work has been done of recent years in different countries, and this seems a fitting occasion upon which to give a brief summary of what is known to-day, especially since the literature relating to symbiosis is largely foreign, somewhat scattered and relatively inaccessible.

I. SYMBIOSIS IN PLANTS

(1) *Lichens*

It is well known to botanists that the vegetative body (thallus) of lichen plants consists of two distinct organisms, a fungus and an alga. The alga, individual elements of which are called "gonidia," is either scattered throughout the thallus or, as in most cases, it forms a well-defined layer beneath the surface of the thallus. The view that lichens consist of the two elements mentioned was advanced by Schwendener (1867–69), who regarded the fungus as living parasitically upon the alga, a view which gained support from the researches of Bornet (1872), Voronin (1872), Treube (1873), etc., and especially of Bonnier (1886–89), wherein synthetic cultures were obtained by bringing together (*a*) various algae obtained in the open and (*b*) fungus-spores isolated from cultures of fungi forming the one component of certain lichens.

[1] Address of the president of the Section of Physiology of the British Association for the Advancement of Science, Liverpool, 1923.

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Schwendener's view, that the fungi are parasitic on the algae in lichens, was contested by Reinke (1873) on the ground that a state of parasitism did not explain the long and apparently healthy life of the associated fungi and algae, a biological association for which the term *Consortium* was proposed by him, that of *Homobium* by Frank (1876), and that of *Symbiosis* by de Bary (1879), the latter term denoting *a condition of conjoint life that is more or less beneficial to the associated organisms or symbionts.*

Investigation has shown that the relation or balance between the associated organisms varies in different lichens. Thus in some forms of *Collemaceae,* as stated by Bornet (1873), the partners as a rule inflict no injury upon each other, whilst in some species of *Collema* occasional parasitism of the fungus upon the alga (*Nostoc*) is observable, since short hyphal branches fix themselves to the alga cells, these swelling, their protoplasm becoming granular and finally being voided. In *Synalissa* and some other lichens the hypha penetrates into the interior of the alga, where it swells and forms a sucker, or haustorium. Elenkin (1902–06) and Danilov (1910) take it as proved that lichens owe their origin to parasitism, the fungus either preying upon the alga or living as an "endosaprophyte" (Elenkin) upon the algae that die.

Therefore, we may find in lichens the condition of true symbiosis on the one hand, ranging to demonstrable parasitism on the other, and, conversely to what has been described above, instances are known wherein algae are parasitic on fungi (Beijerinck, 1890).

Physiology of Lichens

The nutrition of algae in lichens is similar to that of other chlorophyllaceous plants, the most important work on the subject being that associated with the names of Beijerinck (1890) and Artari (1902). In respect to nitrogen supply, Beijerinck cultivated various green algae, as well as gonidia derived from *Physcia parietina.* The gonidia only multiplied rapidly in a malt-extract

culture-medium to which peptones and sugar were added.
This showed that the algae associated with fungi as in
lichens were placed advantageously in respect to nitrogen
supply.   He termed such fungi "ammonia-sugar-fungi,"
because they extract nitrogen from ammonia salts and,
in addition to sugar, form peptones.   Artari showed that
there exist two physiological races in green algae, those
which absorb and those which do not absorb peptones.
He found that the gonidia (*Cystococcus humicola*) de-
rived from *Physcia parietina* absorbed peptones, and he
consequently referred to such algae as "peptone-algae."
Treboux (1912), however, denies the existence of peptone-
sugar-races of algae, and regards the algae in lichens as
the victims of parasitic fungi.   Nevertheless, the impor-
tant researches of Chodat (1913) have demonstrated that
cultivated gonidia develop four times as well when sup-
plied with glycocoll or peptone in place of potassium
nitrate.

The carbon supply of gonidia, according to Artari
(1899, 1901), Radais (1900) and Dufrenoy (1918), is not
derived photosynthetically, but from the substratum on
which they grow.   Whilst Tobler (1911), in his culture
experiments with lichens, found that the gonidia obtain
their carbon from calcium oxalate secreted by the fungus,
Chodat (1913) observed that cultured gonidia grow but
slowly without sugar (glucose), which he believes con-
stitutes their main source of carbon supply.

Whereas, according to Chodat, the gonidia grow poorly
on organic nitrogen in the absence of sugar, they develop
rapidly when sugar is added.   He therefore concludes
that the gonidia lead a more or less saprophytic life in
that they obtain from the fungus-hyphae both organic
nitrogen and carbon in the form of glucose or galactose.

The nutrition of fungi in lichens depends partly upon
parasitism, when they invade the gonidia, and partly
upon saprophytism, when they utilize dead gonidia
(Chodat).

In concluding this section, the hypothesis of M. and

Mme. Moreau (1921) demands mention, since it bears upon the manner in which lichens may have originated in nature. They regard the fungal portion as a gall-structure arising from the action of the associated alga. The lichen, according to this view, is to be regarded as a fungus that has been attacked by a chronic disease which has become generalized and necessary for the subsistence of the host-fungus. F. Moreau (1922) sums up this view as follows: "The lichen-fungus appears as an organism characterized in its morphology by deformity due to an infective agent, an alga. The history of the association existing in lichens may be described as that of a contagious malady marked by the invasion, development, inhibition, and death of the infective agent on the one hand, and on the other hand by the morphological reactions and defensive processes of the attacked organism. In conformity with the virulence and relative immunity of the two opponents, the struggle may be short, the association transitory, the conflict may last indefinitely and the association, rendered lasting, presents the appearance of a harmonious symbiosis."

(2) *The Root-nodules of Leguminous and other Plants*

A well-known example of symbiosis is afforded by the presence of the bacteroids in the nodules of leguminosae, the micro-organisms being capable of fixing atmospheric nitrogen and thereby rendering nitrogen available for assimilation by the plant. This was demonstrated by Hellriegel and Willfahrt (1888), Schloesing and Laurent, whilst Beijerinck cultivated *Bacterium radicicola* from the nodules and produced nodules synthetically by bringing the plant and bacterium together on previously sterilized soil. According to Pinoy (1913), the bacteroids are myxobacteria, and, in the case of one species which he has especially studied (*Chondromyces crocatus*), it was found essential for the successful cultivation of the micro-organism, apart from its host-plant and in vitro, that it should be grown in association with a species of

micrococcus; similar observations have been made on other micro-organisms by bacteriologists, and some refer to the conditions as one of symbiosis. Bacteriologists, I would note, are continuously misapplying the term symbiosis in referring to bacteria grown in mixed cultures, when there is no evidence whatever that the micro-organisms are mutually interdependent for their growth. In passing, it may be mentioned that nodules on the roots of the alder are attributed to the presence therein of *Streptothrices,* and that comparable nodules occur in Eleagnaceae. The nodules on the leaves of Rubiaceae and tropical Myrsinaceae are also regarded as due to bacterial symbionts.

(3) *The significance of Mycorhiza in relation to various Plants*

It has long been known that the roots of *most perennial and arborescent plants* are invaded by the mycelium of fungi known as *Mycorhiza,* and it is to Kamiensky (1881), and especially Frank (1885), to whom we owe the hypothesis that we are here dealing with symbiotic life. Frank distinguishes two forms of *Mycorhiza*: (1) the *ectotrophic,* which surround the root externally like a sleeve and are found especially about the roots of forest trees (Conifers), and (2) *endotrophic,* which penetrate deeply into the root tissue and even into the cells of the root. The *endotrophic Mycorhiza* are derived from the outside; their mycelium enters the root by penetrating the epidermal cells at the base of the root hairs, passes between the cells and into them where the mycelium branches dichotomously, and forms ultimately a much-branched intracellular growth. By this time the fungus is no longer in communication with the exterior of the root, and it nourishes itself within the host cell, only, however, by utilizing the reserve substances stored there whilst avoiding the cell protoplasm or other living host elements. The host cell, after a period of inertia, exhibits a distinct reaction to the presence of the fungus, in that its nucleus becomes hypertrophied, divides repeatedly and

becomes amoebiform in contour. The contained mycelial mass undergoes degeneration, is digested by the host, and the host-cell resumes its normal life. These root-*Mycorhiza* have not as yet been cultivated,[2] as have others to which reference will presently be made, and it is as yet impossible to assign them a place among known species of fungi. Further details regarding these forms will be found in the publication of Gallaud (1904).

Mycorhiza in Orchids

The first to note the presence and to attempt to cultivate the fungus mycelium in the roots of orchids was Reisseck (1846), and in 1881 Kamienski advanced the hypothesis that the association was one of symbiosis. Wahrlich (1889) subsequently found symbionts in all species of orchids he examined, about 500 in number, thereby showing that their distribution is generalized.

It is to the researches of Noël Bernard (1902 onward), however, that we are actually indebted for the complete demonstration of the true relation existing between orchids and *Mycorhiza,* based as it is upon physiological studies. All who had to do with orchids in the last century found the greatest difficulty in raising these plants from their seed; a successful result appeared to depend largely on chance. Cultivators of orchids found that success was obtained more frequently by placing seed in soil upon which orchids had previously lived, and much secrecy was observed as to the methods employed by the more successful cultivators.

The seeds of orchids are exceedingly small—a million may be found in a single capsule of an exotic species; they possess no albumen and contain an embryo consisting merely of a mass of undifferentiated cells provided with a suspensor. The essential discovery of Bernard was that orchid seeds do not germinate in the absence of fungi belonging to the genus *Rhizoctonia.* The fungus

[2] Magrou (1921) reports that he isolated *Mucor solanum* n. sp. from *Solanum dulca-mara,* and he seems to have infected the potato plant with the fungus.

enters the seed through its least resistant and highly permeable cells, which apparently emit a secretion that attracts the fungus. Each species of orchid, according to the subsequent researches of Burgeff (1909), possesses a special species, variety or race of fungus that is particularly adapted to it—he distinguishes fifteen species of fungus. When mutually adapted orchid seed and fungus are brought together, the mycelium of the latter penetrates the suspensor cells by digesting their cellulose wall. The mycelium traverses the epidermal cells of the seed without undergoing development within them. As soon as the primary infestation has occurred, even where the mycelium has penetrated but slightly, the cells of the seed, situated at the posterior pole of each embryo, cease to be vulnerable. In other words, a local immunity appears to be established, this immunity lasting at any rate until new regions are attacked by the fungus. This, in Bernard's experience, is the general rule. The mycelium, having attained the parenchyma cells, develops into characteristic filamentous masses recalling the appearance seen in bacterial agglutination. Nevertheless, there comes a time, this varying according to the associated species involved, when the development of the fungus is arrested by the deeper parenchyma cells of the seeds. These cells are altered before they are penetrated by the fungus; they become hypertrophied and acquire large lobose nuclei. They digest the mycelium which enters their protoplasm, but the cell continues to harbor remains of the fungus ("corps de dégénérescence") which occur abundantly in the tissues of orchids. The seed now proceeds to sprout, giving rise to a small tubercle ("protocorm"), which only at a later period produces leaves and roots.

The cultivation of Rhizoctonia of various species was carried out successfully by Bernard, the cultures being used to reproduce germination in orchids. Orchid seeds alone remained unchanged for months in cultures on agar with salop-decoction added, but when pure cultures of

Rhizoctonia mycelium were added to such orchid seeds, the latter were invaded by the fungus, germinated, and gave rise to a "protocorm." Bernard gives excellent figures illustrative of the development described.

The relation between the fungi and orchids varies in different groups and plants. In primitive forms like *Bletilla* germination occurs in the absence of the fungus, but the "protocorm" does not develop; the rhizome, to which the plant is periodically reduced, is only periodically attacked when fresh roots are formed. *Bletilla*, however, behaves in an exceptional manner. In other orchids (*Ophrydeae, Cattleyeae, Cypripedeae,* etc.) the fungus is needed for germination, and the adult plant is fungus-free except when the orchid produces fresh roots. Therefore, in such cases symbiosis is intermittent. In higher orchids like the epiphytic *Sarcanthineae* the fungus is needed for germination, and, the roots being persistent, symbiosis is maintained continuously. Finally, in *Neottia nidus-avis* the symbiotic condition is maintained throughout the life-cycle of the orchid, the fungus being found in the roots, rhizome, and even in the flowers and seeds, and it is transmitted hereditarily.

The activity or "virulence" of Rhizoctonia, according to Bernard, diminishes when the fungus is kept apart from the orchid, being practically lost after two or three years. An attenuated fungus regains its activity in a measure after a sojourn of some weeks in a young orchid plant; a full degree of activity under symbiotic conditions is, however, only regained slowly.

The germination of orchids in the absence of fungi was successfully induced by Bernard through cultivating them in concentrated nutrient solutions of a kind that does not occur in nature; such solutions, moreover, except under carefully carried out experimental conditions, would be rapidly vitiated through serving as a medium for the multiplication of different micro-organisms. The effect of increasing the concentration of the solution, offered to plants reared without fungi, corresponds to that

obtained by raising plants with fungi of increasing activity or "virulence." It may be added here that when *Rhizoctonia* are cultivated on a medium containing saccharose and the substance of orchid tubers—namely, salop—they cause an increase in the molecular concentration of the medium. It is possible that the fungi, when associated with the orchids, bring about a similar increase in the molecular concentration of the sap of the invaded plant.

The Origin of Tubers in Various Plants

The occurrence of endotrophic *Mycorhiza* in the roots of species of *Solanum* has been recorded by Janse (1897) for *S. verbascifolium* in Java, by Bernard (1909–11) for *S. dulca-mara,* by Mme. Bernard and Magrou (1911) for *S. maglia* collected in Chili, the last-named species having been regarded by Darwin as the wild type of *S. tuberosum,* our edible potato.

Experimenting with the potato, Molliard (1907, 1920) found that tubers were not formed in aseptic cultures in a poor nutrient medium, and that raising the concentration of the sugar in the sap artificially (as with the radish) led to tuberization; concentrating the culture-medium did not induce tubers. Magrou (1921) placed potato seeds in a poor soil and close to *S. dulca-mara,* which always contains fungi, and found that only when the fungus invaded the potato plant were tubers formed.

Magrou also investigated tuberization in *Orobus tuberosus* (Leguminosae) and in *Mercurialis perennis* (Euphorbiaceae), and from his collective studies the following conclusions may be drawn:—

(1) When the potato plant and *Orobus* are raised from seed, the establishment of symbiosis leads to tuberization of the sprouts at the base of the stem; tubers are not formed in the absence of symbionts. (2) Owing to developmental differences between the two plants, symbiosis in the potato plant is intermittent, whilst in *Orobus* it is continuous. (3) It follows that these plants may develop in two ways: (*a*) when they harbor symbionts they

produce perennial organs; (*b*) without symbionts they
are devoid of perennial organs.   (4) It is the rule for
wild perennials to harbor symbionts, as Bernard has
stated, whilst annuals are devoid of symbionts; three
species of annuals (*Solanum nigrum, Orobus coecineus*
and *Mercurialis annua*) may be penetrated by endo-
phytes, but they quickly digest the intruders.   (5) These
observations confirm and supplement the view held by
Bernard that tuberization is due to the association of
fungi with plants.

Mycorhiza in Ericaceae

Rayner (1915–16) finds that *Mycorhiza* are constantly
present in heathers.   He isolated *Mycorhiza* (of the
genus *Phoma*) from *Calluna vulgaris,* in which the fungus
is widely distributed, being found in the roots, branches
and even in the carpels, so that it occurs within the ripe
fruit and seed tegument.   *Calluna* seeds, when grown
aseptically, give rise to poor little plants devoid of roots,
but, under like conditions, in contact with *Phoma* the
plants develop normally and form many roots.

Mycorhiza in Club-mosses and Ferns

In Lycopodiaceae (Club-mosses) and Ophioglossaceae
(Ferns), according to Bernard, the perennial prothallus
is infested, and the spores whence the plants emanate will
not germinate except (as with orchid seeds) with the
help of fungi.

In concluding this part of my subject, dealing with
symbionts of plants, I need scarcely emphasize the sig-
nificance of symbiosis in the vegetable kingdom.   I will
close by mentioning the theoretical deduction of Bernard
that vascular plants owe their origin in the past to the
adaptation of certain mosses to symbiotic life with fungi.

II. Symbiosis in Animals

(1) Algae as Symbionts

Animals of widely separated groups characterized by
their green color have long been known.   Already in

1849, von Siebold attributed the color of *Hydra viridis* to chlorophyll which, for a period, was regarded as an animal product. In 1876, Gésa Entz concluded that the chlorophyll is contained in vegetable cells living as parasites or commensals within the animals; these cells were aptly named *zoochlorella* by Brandt (1881), whilst cells distinguished by their yellow color were subsequently called *zooxanthella,* the latter having been first described by Cienkovsky (1871) as present in Radiolaria. In the latter case the symbionts were found capable of surviving their host, of multiplying and of assuming a flagellate stage.

Zoochlorella occur mainly in fresh-water animals, zooxanthella mainly in marine animals, the symbionts, measuring 3–10 microns in size, being found in many Protozoa, Sponges, Coelenterates, Ctenophores, Turbellaria, Rotifers, Bryozoa, Annelids and Molluscs.

Physiological relations between Animals and Symbiotic Algae.—In 1879, Geddes showed that green animals give off oxygen, *Convoluta roscoffensis* (Turbellaria), when well illuminated, liberating gas containing 45–55 per cent. of oxygen. Engelmann (1881), by means of his bacteria-method, showed that *Hydra viridis* (Coelenterata) and *Paramaecium bursaria* (Protozoa) give off oxygen when exposed to light. Geddes (1882), working with a series of marine animals, found *Velella* gave off 21–24 per cent. of oxygen, and an Actinia (*Anthoa cereus*) gave off 32–38 per cent. of oxygen. Whereas animals harboring green algae as symbionts always liberated oxygen, the colorless varieties of these animals never did so. Geddes regarded the association of animal and alga as being mutually helpful, the oxygen supplied by the alga to the animal and the carbon dioxide and nitrogen supplied by the animal to the alga being useful to the partners. He speaks of "animal lichens" and "Agricultural Radiolarians and Coelenterates." He found, moreover, that animals harboring symbionts are much more resistant than those without symbionts:

Medusae (*Velella*) survived 14 days in small beakers with symbionts, only 1–2 days without them. Protozoologists have, moreover, found that Protists harboring symbionts are easier to rear in vessels than are those without symbionts. Brandt (1883) believes that the symbionts and host aid each other in nutrition. Green *Spongilla* (fresh-water sponges) and *Hydra viridis* may live a long time in filtered water. He found that when starved green Actinia were (*a*) placed in the dark, they expelled their algae and died rapidly, being probably poisoned by the dead algae, but that when they were (*b*) placed in diffuse light they lived on. Actinia deprived of symbionts may become habituated in culture to live without them. Opinions (*vide* Buchner, 1921) are in conflict as to the exact relationship between the partners; in some cases (*Peneroplis* and *Trichosphaerium*) the symbionts never appear to be injured, in *Amoeba viridis*, etc., a limited number of symbionts are digested at all times, whereas in some Radiolarians, etc., digestion only takes place at certain stages of their development. Nutritive substances pass from the algae into the host's cells; thus starch granules, found alongside the algae, may be taken up by the animal cells.

Using modern methods of gas analysis, Trendelenburg (1909) concludes that green Actinias (*Anemonia sulcata*) live in true symbiosis with algae, the algae supply oxygen to the animal by day and at night utilize the surplus oxygen evolved, whilst carbon dioxide is furnished to the alga partly by the animal and partly by the water in which they are bathed. Rütter (1911) studied the nitrogen metabolism and concludes (*a*) that the Actinia yields to the algae nitrogen in the form of ammonia for protein synthesis, and in darkness it adds carbon containing substances (nitrogen-free), whilst (*b*) the algae yield to the Actinia nitrogenous substances in the dark and by light carbon-containing substances. Organisms harboring algae exhibit naturally a positive heliotropism.

Symbiotic algae are not usually transmitted heredi-

tarily, each host-generation being usually infected afresh by algae, encountered about the host, which may be either free-living or possess a free-living stage in their development. Exceptions occur, however, where Protozoa multiply by division and the algae pass directly (as it were hereditarily) to succeeding generations. There are also cases of hereditary transmission in hosts that undergo sexual multiplication (as in *Hydra viridis*), the zoochlorella penetrating the egg on escaping from the host's endodermal cells after the manner of starch granules or other food reserve substances (v. *supra*). From the circumstances that in most cases symbiotic algae are not transmitted hereditarily, we may explain the occasional occurrence of alga-free individuals in a species usually harboring the symbionts.

Studies conducted on Turbellaria are of special interest: These animals may contain either green or yellow symbionts, and, as in Protozoa, some allied species harbor the symbionts and others do not. The eggs of Turbellaria are symbiont-free, each generation becoming infected afresh, the symbiont either entering the host's mouth and remaining there, traversing the intestinal wall, or entering by the genital pore, according to the particular host-species it affects.

The best-known example of symbiosis in Turbellaria is found in *Convoluta roscoffensis,* a species that has been well studied by Keeble and Gamble (1903–07). Its larvae are colorless and infection occurs after hatching. Colorless larvae are obtainable by transferring freshly hatched examples immediately to filtered sea-water. The cocoon, on the day following its deposition, is already invaded by many algae having a very different structure from those found in *Convoluta*; they possess four flagella and have been referred by Keeble and Gamble to the genus *Carteria* (allied to *Chlamydomonas*). The algae within the host possess a special structure, their contour is very irregular, they have no cellulose wall, the green coloring matter is unevenly distributed, being confined to chro-

matophore bodies surrounding the pyrenoid body, the nucleus is eccentric, and a number of examples are found with degenerating nuclei. Naturally all attempts to cultivate these obviously degenerating algae have failed.

The physiological relations existing between Turbellaria and algae differ according to the species. Thus in *Vortex viridis* symbiosis is not necessary, in *Convoluta* it is necessary for both partners. Mature *Convoluta* are never found devoid of algae in nature. The young larva can only feed itself for a week; as it grows older it becomes infected progressively with algae and ceases to nourish itself otherwise than upon the products of its contained symbionts. Finally, having reached an advanced age, the animal becomes capable of digesting the algae, as does *Convoluta paradoxa* under unfavorable conditions of life. Keeble and Gamble define four periods in the life of *Convoluta,* which they term respectively hetero-, mixo-, holo-, and auto-trophic, wherein the animal lives at the expense (1) of formed substances, (2) of these and alga-products, (3) of alga-products only, and finally (4) of the algae themselves. This constitutes a true evolution in a species from a free existence, depending only on outside sources of food supply, to a symbiontic mode of life, and lastly one merging into parasitism.

(2) *Symbiosis in Insects*

Among insects we find a whole series of progressive adaptations toward an association with micro-organisms of different categories:

Group I.—*The utilization by insects of micro-organisms cultivated by them outside their bodies.* To quote three examples: (1) The larvae of the beetle *Xyloteres lineatus* (Bostrichidae) form galleries in the wood of Pines. The galleries have a characteristic blue color, produced by the growth of the fungus *Ambrosia* upon their walls, the fungus being cultivated by the larva for food. The beetle is incapable of digesting cellulose. Analogous cases occur among Ants and Termites thus:

(2) *Termes perrieri* of Madagascar, studied by Jumelle and Perrier de la Bâthie (cited by Portier, 1918), builds numerous chambers and galleries. The termites collect dead wood, chew it up finely, swallow it, the wood passing unaffected through their intestine and out in the form of small spherical masses (0.5 mm) which are cemented together as porous cakes that are impregnated with digestive secretions. Fungi which develop upon the cakes serve as food for the termites, and in well-cared-for nests the growth is harvested by the workers who triturate the mycelium and spores and feed the young larvae therewith, whilst older larvae receive spores, and large larvae receive mycelium and the triturated wood contained in the cakes. (3) A third example is that of ants belonging to the genus *Atta* which cultivate fungi over areas of 5 to 6 square meters; here the queen, when about to found a new colony, carries away a small ball of fungus in a corner of her mouth wherewith to start a fresh culture in the new habitat.

Group II.—*Symbiotic organisms developing in the lumen of the intestine and its adnexa.* As examples may be cited the bacteria occurring in the intestines of fly larvae (*Musca, Calliphora*, etc.), which aid the larva to digest meat; the bacteria associated with the olive-fly (*Dacus olea*); the Trychonymphids of xylophagous Termites (*Leucotermes lucifugus*).

Group III.—*Intestinal symbionts situated in the epithelial cells of the digestive apparatus.* The most striking instance is found in *Anobium paniceum,* a small beetle commonly occurring in flour, biscuits, dried vegetables, etc. In a part of its mid-gut are found cells filled with symbiotic yeasts undergoing multiplication (Escherich, 1900). The symbionts are not transmitted hereditarily but are acquired by the larva on hatching, being eliminated by the female beetle.

In this connection may be mentioned with reserve the observation of Portier (1918) upon xylophagous Lepidoptera (*Cossus, Nonagria, Sesia,* etc.) which, according

to that author, possess intestinal fungi (*Isaria*) that multiply in the gut and form spores that penetrate the intestinal epithelium and attain the perivisceral cavity, fatbody and muscles of the insect. As Caullery points out, however, the supposed spores closely resemble Microsporidia, and Portier's interpretation may be erroneous. In this category also belong the symbionts described as occurring in *Glossina* by Roubaud (1919) and before him by Stuhlmann, these being found in certain hypertrophied cells of the intestinal epithelium. When liberated into the gut lumen, the symbionts are stated to multiply by budding after the manner of yeasts. Roubaud regards the yeasts as fungi, allied to the *Cicadomyces* of šulç, and finds that they are transmitted hereditarily from the adult to the egg, larva and pupa.

Group IV.—*Intracellular symbionts of deep tissues.* This group of symbionts is most frequently found in insects, but their nature was not disclosed until recent years. Already, in 1858, Huxley described an organ which is constantly present close to the ovary in *Aphis*. Balbiani (1866) named it the pseudovitellus, or green body, and Metchnikoff (1866), who followed its development, named it "secondary vitellus." The function and structure of this organ were studied by subsequent authors without being understood until, in 1910, there appeared two important papers by Pierantoni (February 6), and šulç (February 11), who demonstrated their symbiotic character, recognizing the intracellular inclusions as yeasts whose evolution they completely followed. Their results have been confirmed by various authors, especially by Buchner, who in a remarkable series of papers describes a number of associations existing between insects and micro-organisms and reaches important generalizations as to their significance. It is from a collective work on the subject by Buchner (1921) that most of our information regarding this class of symbionts is taken.

Among the symbionts of deep tissues in insects are

found a whole series of specializations among the host-elements harboring the symbionts. The least specialized instance is represented by Lecaniinae where the yeasts are distributed throughout the body (perivisceral fluid, cells of fat-body); the fat-body cells may be regarded here as facultative Mycetocytes. In cases like *Orthezia,* symbiotic bacteria occur in certain fat cells which still contain fat droplets; this condition is also found in certain Cicadas, the yeasts being contained in fat cells which continue to accumulate fat, glycogen and urates. Finally cases occur as in Blattids where symbiotic bacteria are found in special cells greatly resembling fat cells but already forming well differentiated Mycetocytes. This class is well represented in and about the digestive tract of Pediculidae (*Haematopinus*) and certain ants (*Camponotus*). Still more advanced in specialization are those cases in which the symbiont-containing cells (*Mycetocytes*) agglomerate to form true organs termed *Mycetomas,* organs that are surrounded by flattened epithelial cells, the component mycetocytes containing either yeasts or bacteria as symbionts; such cases are found in Aphids, Chermids and Aleurodids. Mycetomas may occur singly or in numbers according to the nature of the host; the epithelial covering of the organ varies in its cell structure and pigmentation and the organ may be plentifully supplied with tracheae whose finest branches penetrate into the interior of the mycetocytes. The relations between the mycetocytes or mycetomas and the other organs of the host vary greatly; in some cases they occur especially in the fatty tissue, in others near the gonads, in others, as in Pediculidae, around or upon the intestine. In *Pediculus* and *Phthirus,* parasitic on man, the mycetoma is disc-shaped and lies centrally as a distinct milk-white structure upon and indenting the mid-gut. Transition forms between isolated mycetocytes and differentiated mycetomas are found in various insects.

The mode of transmission of intracellular symbionts of insects from generation to generation may take place in different ways as defined by Buchner (1921, somewhat modified):

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I. The larva of each generation infects itself through the mouth (Anobiidae).

II. Infection takes place hereditarily through the egg:

    1. By symbionts set free in the blood, or which leave mycetocytes or mycetomas and attain the egg as follows:

        (a) by general infection of follicles and invasion of the egg, and finally establishing themselves at the posterior pole of the egg (Ants);

        (b) by penetrating special parts of the follicles, producing for a period bacterial vegetation upon the whole egg and finally concentrating at the egg's two poles (Blattidae);

        (c) by entering the egg *via* its nutritive cells

            (a) only some isolated fungi entering (Lecaniinae);

            (b) a number of bacteria enter in the form of a gelatinous mass (Coccinae);

        (d) by entering the posterior pole of the egg:

            (a) as isolated fungi

                (a) which penetrate one after another (Aphids);

                (b) which accumulate in follicles and enter in a mass consisting of

                    (a) one kind of symbiont (Icerya);

                    (b) two kinds of symbiont (Cicada, Aphrophora);

                    (c) three kinds of symbiont (Aphalara ?);

            (b) as bacteria united in several gelatinous masses (Orthezia).

    2. By whole mycetomas entering at posterior pole of egg (Aleurodes).

    3. By isolated symbionts leaving special mycetomas situated at juncture of follicular tubes (Pediculidae).

III. Embryonal infection as in parthenogenetic Aphids.

It is difficult to understand the mechanism whereby the symbionts penetrate the egg in the insect's body; in any case the complicated procedure must depend upon a mutual and parallel adaptation of the insects and microorganisms concerned.

During embryonal development the topographical distribution of the mycetocytes varies from one group of insects to another. In *Camponotus* they occur dorsally upon the mid-gut; in Blattidae the bacteria are at first localized in the intestinal lumen, passing thence through the intestinal epithelium and entering the fat-cells. In Hemiptera and Pediculidae the symbionts form a mass at the posterior pole of the germinal layer, and during ver-

sion or unrolling of the embryo they penetrate in the
ventral region of the abdomen.

As already indicated, the symbionts may be Yeasts,
Saccharomycetes, Bacteria, or even Nitrobacteria. Their
entrance into the cells and their presence therein even in
large numbers does not in many cases prevent multiplica-
tion of the invaded cells or affect their mitosis; in other
cases mitosis is more or less affected; it may become mul-
tipolar and lead to synsytium formation; and finally,
cases may occur in which mitosis ceases and the symbi-
ont-bearing cells divide amitotically.

We know little regarding the part played by symbionts
in insects; our information relates almost exclusively to
their morphology, mode of multiplication, and entry into
the host during its development. There are no indica-
tions that the symbionts are injurious or pathogenic. It
is evident, however, that they find in certain insects fa-
vorable conditions for growth, multiplication and trans-
mission from host to host. In these cases, therefore, we
are dealing with a constant very harmonious association
which excludes even a suspicion of there being any con-
flict between the associated organisms. We may well ask
ourselves what are the reciprocal advantages of this as-
sociation, but this is a question that it is impossible to
answer in view of our ignorance of physiological and bio-
chemical processes in insects.

Various hypotheses have been advanced to explain the
possible function of the symbionts. Symbiotic yeasts
may decompose urates (šulç); they may produce an en-
zyme that aids in digestion of sugars, as in Aphids (Pier-
antoni); they may aid in digestion of cellulose in xylo-
phagous insects which alone can not render cellulose as-
similable (Portier); the Nitrobacteria found in various
Hemiptera may fix free nitrogen which is conveyed to
them through the host's tracheae, and thus supply the
host with nitrogenous substances, thereby meeting a de-
ficiency in its food supply.

Phytophagous Hemiptera nourish themselves chiefly

upon leaf-sap without utilizing the protoplasm of the
plant-cells they penetrate with their sucking mouthparts.
The imbibed sap is rich in mineral substances, carbohy-
drates and glycosides only. In these insects Peklo finds
two different symbionts, Saccharomycetes and coccoid
organisms, whilst Pierantoni attributes to symbionts the
pigment production in *Coccus cacti.*

(3) *Micro-organisms in Relation to Luminescence in Animals*

A fairly large number of organisms are known which
have the faculty of emitting light. They are found among
Bacteria, Fungi, Protozoa, Coelenterates, Echinoderms,
Worms, Molluscs, Crustacea, Insecta, Tunicata and Fish.
As a rule luminescence in animals depends upon the ac-
tion of luciferase on luciferin, but recently a number of
cases have become known wherein light production has
been traced to micro-organisms, and it is with these cases
that we shall deal.

Luminescent pathogenic bacteria may invade the host,
as described by Giard and Billet (1889–90), for the small
marine amphipod, *Talitrus,* of which rare light-emitting
examples may be found in nature. The affected crusta-
cean dies in about six days. The pathogenic bacterium
does not luminesce in cultures, but does so when inocu-
lated into *Talitrus.*

Luminescent symbiotic bacteria present in various
light-emitting animals are, however, of direct interest to
us, since their presence has been determined in lumines-
cent organs of certain insects, cephalopods, tunicates and
fishes:

Insects: Pierantoni (1914) investigated the luminous
organs of glow-worms (*Lampyrus*), and found them to
consist of parenchyma cells crowded with minute bodies
having bacteria-like staining reactions, these bodies being
also present in the beetle's egg, which is luminous. He
cultivated two species of micro-organisms from the or-
gans, but does not distinctly establish their causal rela-
tionship.

CEPHALOPODS: We owe to Pierantoni (1917–20) and Buchner the discovery that luminescence in certain Cephalopods is due to light-producing bacterial symbionts living in special organs of the host. These organs may be simple or otherwise. In *Loligo* the luminous organs, hitherto known as "accessory nidamentary glands," represent the simpler type of organ, this consisting merely of a collection of epithelial tubes surrounded by connective tissue. In cuttle-fish (*Sepiola* and *Rondeletia*) the organs are more complicated, the glands being backed by a reflector, and provided outwardly with a lens serving for the projection of the light rays generated by the symbionts within the tubes. The symbionts are transmitted hereditarily when the Cephalopods lay their eggs. The symbionts of *Loligo* and *Sepiola* have been cultivated by Pierantoni and Zirpolo (1917–20); they inhabit the gland-tubes of the luminescent organs in large numbers, and produce light continuously, as do other luminescent bacteria in cultures.

TUNICATA; The Pyrosomidae, all of which emit light and form tubular colonies, have long attracted the attention of biologists. Each individual in the colony possesses two fairly large luminescent organs, whose structure was studied by Panceri (1871–77), Kovalevsky (1875), and especially Julin (1909–12), who observed in the cells of the luminous organ riband-like structures appearing knotted here and there. Julin regarded the structures as mitochondria or chromidia, and it was left to Buchner (1914) to explain their true nature; they are symbiotic fungi, and are transmitted hereditarily. Buchner gives a detailed study of the symbiont and a review of the physiology of luminescence and of Pyrosomes which is well worth consulting by those interested in such problems.

FISH: Of great interest are the researches of Harvey (1922) upon light production by two species of fish (*Photoblepharon* and *Anomalops*) which occur in the sea about the Banda Islands, Moluccas. Their life-history is

unknown. They measure up to about 11 cm in length.
The author writes: "In both fishes the luminous organ is
a compact mass of white to cream-colored tissue, flattened
oval in shape, lying in a depression just under the eye and
in front of the gills. The organ looks as if made for ex-
perimentation, as it is attached only at the dorso-anterior
end, and can be cut out with the greatest ease, giving a
piece of practically pure luminous tissue. The back of
the organ is covered with a layer of black pigment, which
serves to keep the light from shining into the tissues of
the fish. In both fishes there is a mechanism for obscur-
ing the light, but, curiously enough, the mechanism de-
veloped is totally different in the two species, notwith-
standing the fact that in structure the organ is identical
in the two, and in every detail except proportion the
fishes are very similar. In *Anomalops* the organ is
hinged at the antero-dorsal edge, and can be turned down-
ward until the light surface comes in contact with a fold
of black pigmented tissue, forming a sort of pocket. The
light is thus cut off. In *Photoblepharon* a fold of black
tissue has been developed on the ventral edge of the
organ socket, which can be drawn up over the light sur-
face like an eyelid, thus extinguishing the light." The
histological structure of this organ was worked out by
Steche (1909). The organ is continuously luminous day
and night, and independent of stimulation. According
to Steche, *Anomalops* constantly turns the light on
and off (10″ light, 5″ dark), the fish using it, he supposes,
as a searchlight to attract and mislead its prey. The
natives use the amputated organ as a bait in night fish-
ing; it maintains its luminosity for about eight hours.
The organ is described by Steche as composed of a great
number of sets of parallel gland tubes (acinose), sepa-
rated by connective tissue, and extending across the or-
gan from the back pigmented surface to the front trans-
parent surface, each set arranged in a ring about a vessel
which provides them with blood and oxygen. Near the
surface a number of these tubes unite into a common res-

ervoir, opening outward through a minute pore which admits sea-water. A number of pores dot the surface of the organ. The luminous material fills the lumen of the tubes; it is extracellular but intraglandular, and is never voided from the gland. Harvey states that the luminous material filling the tubes consists of an emulsion containing many granules and rods; the latter move about with a corkscrew-like motion, and are undoubtedly bacteria. The luminosity of the organ is due to these symbiotic bacteria. An emulsion containing the symbionts behaves exactly like an emulsion of luminous bacteria in being sensitive to lack oxygen, desiccation, bacteriolytic agents, potassium cyanide, etc. The continuity of the light, independently of stimulation, is characteristic of luminous bacteria and fungi alone among organisms; this, and the circumstance that luciferin and luciferase could not be demonstrated, all go to confirm the correctness of Harvey's conclusions regarding the cause of luminosity in these fish, notwithstanding that he has failed hitherto to cultivate the bacteria found in the luminous organs.

In concluding this section dealing with light production by animals it may be repeated that we have to distinguish between (a) luminescence due to symbiotic organisms, such luminescence being continuous in the presence of oxygen as in cultures of luminous bacteria (of which some thirty species are known), and (b) that due to animal cell-products known as luciferin and luciferase which are secreted and expelled *at intervals,* in response to a stimulus, from two kinds of gland cells, the secretions, when mixed, producing light.

Portier's Hypothesis

The numerous cases in which symbiosis occurs in nature have naturally led some biologists to ask if symbiosis is not a phenomenon of general significance, and perhaps essential, in living organisms. In this connection reference must be made to the hypothesis advanced by Portier (1918), because it formulates extreme views. Starting from his studies of symbionts of leaf-mining

caterpillars (*Nepticula*) and wood-devouring insect larvae (*Cossus, Sesia,* etc.), he sought to verify the work of Galippe (1891–1918) on micro-organisms occurring in vertebrate tissues. Using methods he supposed to be adequate, Portier claimed that he could isolate various micro-organisms from vertebrate tissues. On faulty premises he built up a hypothesis that may be likened to a house of cards. He divides living organisms into two groups, *autotrophic* (bacteria only) and *heterotrophic* (all plants and animals), according as they are provided or not with symbionts. Whereas some symbionts are cultivatable, others have become so domesticated in respect to their hosts that they can not be separated from them. The essential function of symbionts is to elaborate reserve substances so that they become assimilable to the host cell. The mitochondria that are present in all plant and animal cells, though not cultivatable, are, according to Portier, nothing but symbionts, the importance of their function having recently been revealed by Guillermond, Dubreuil and others.[3] They are derived from food, and, if absent therefrom, illness supervenes, as shown by the bad effects of sterilized food, decorticated rice, etc., causing deficiency diseases attributed to lack of vitamines, which, according to Portier, are nothing but symbionts. Where, as in *Aphis,* the animal feeds on plant sap that is filtered through a tube formed by the insect's saliva—in other words, the insect imbibing food devoid of symbionts—the animal is of necessity provided with its own well-developed store of them. Portier applies his hypothesis to such varied problems as fecundation, parthenogenesis, tumor-formation, variation and origin of species, in all of which mitochondria, that is, his supposed symbionts, play a part. His views aroused great controversy in France, so much so that it was thought necessary for the Société de Biologie de Paris (see C. R. Soc. Biol., LXXXIII, 654,

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[3] Guillermond has shown that the mitochondria of the epidermal cells in *Iris* elaborates amyloplast and finally starch. Dubreuil (1913) found that mitochondria elaborate the fat in fat-cells. Other cytologists have shown that glandular secretions are similarly referable to mitochondria.

May 8, 1920) to have a Committee examine the evidence.
The Committee, consisting on the one part of Portier
and Bierry, and on the other of Martin and Marchoux
(Institut Pasteur), by its report indicates the pitfalls,
well known to bacteriologists, into which Portier was led,
and thus disposes of the greater part of his far-reaching
hypothesis. Nevertheless, like many exploded hypotheses, that of Portier has served a useful purpose through
the discussion it has provoked and the interest in the subject of symbiosis which it has stimulated.

CONCLUSION

The term "symbiosis" denotes a condition of conjoint
life existing between different organisms that in a varying degree are benefited by the partnership. The term
"symbiont," strictly speaking, applies equally to the
partners; it has, however, come to be used also in a restricted sense as meaning the microscopic member or
members of the partnership in contradistinction to the
physically larger partners which are conveniently termed
the "hosts" in conformity with parasitological usage.

The condition of life defined as symbiosis may be regarded as balancing between two extremes—complete
immunity and deadly infective disease. A condition of
perfect symbiosis or balance is realized with comparative
rarity because of the many difficulties of its establishment in organisms that are either capable of living independently or are incapable of resisting the invasion of
organisms imperfectly adapted to communal life. In
these respects the conclusions of Bernard and Magrou in
relation to plants apply equally to animals. It is difficult
to imagine that symbiosis originated otherwise than
through a preliminary stage of parasitism on the part of
one or other of the associated organisms, the conflict between them in the course of time ending in mutual adaptation. It is, indeed, probable that some supposed symbionts may prove to be parasites on further investigation.

In perfect symbiosis the associated organisms are completely adapted to a life in common. In parasitism the

degree of adaptation varies greatly; it may approach symbiotic conditions on the one hand, or range to vanishing point on the other by leading to the death of the organism that is invaded by a highly pathogenic animal or vegetable disease agent. There is no definite boundary between symbiosis and parasitism. The factors governing immunity from symbionts or parasites are essentially the same.

No final conclusions can as yet be reached regarding the function of symbionts in many invertebrate animals owing to our ignorance of the physiological processes in the associated organisms. The investigation of these problems is one fraught with difficulties which we must hope will be surmounted.

New knowledge is continually being acquired, and a glance into new and even recent publications shows that symbionts have been repeatedly seen and interpreted as mitochondria or chromidia. Thus in *Aphis* the long-known pseudovitellus has been shown to contain symbiotic yeasts by Pierantoni and Šulç, independently and almost simultaneously (1910); Buchner (1914) has demonstrated symbiotic luminescent fungi in the previously well-studied pyrosomes, besides identifying (1921) as bacterial symbionts the mitochondria found by Strindberg (1913) in his work on the embryology of ants. The increasing number of infective diseases of animals and plants, moreover, which have been traced, especially of recent years, to apparently ultramicroscopic organisms, can not but suggest that there may exist ultramicroscopic symbionts.

From the foregoing summary of what is known to-day of symbiosis we see that it is by no means so rare a phenomenon as was formerly supposed. Symbiosis occurs frequently among animals and plants, the symbionts (Algae, Fungi, Bacteria) becoming in some cases permanent intracellular inhabitants of their hosts, and at times being transmitted from host to host hereditarily. Among parasites, non-pathogenic and pathogenic, we

know of cases wherein hereditary transmission occurs from host to host.

It is evident that we are on the threshold of further discoveries, and that a wide field of fruitful research is open to those who enter upon it. In closing, it seems but fitting to express the hope that British workers may take a more active part in the elucidation of the interesting biological problems that lie before us in the study of symbiosis and the allied subject of parasitism.

SHORTER ARTICLES AND DISCUSSION

THE ABSENCE OF LUTEAR CELLS IN THE TESTIS OF THE MALE PHALAROPE

CERTAIN experiments that I carried out several years ago with sebright bantams, in which breed of fowls the plumage of the male is like that of the female, showed that the suppression of the cock-feathering of the male is due to some hormone produced by the testis. It is known that a hormone produced in the ovary of the female of other races of domestic fowls also inhibits the development of cock-feathering in the female. Whether this substance is the same as that produced in the testis of the male sebright has, however, not been shown.

In the testis of the male sebright certain cells are present that resemble the so-called lutear cells of the ovary of the female sebright, and of the ovary of females of ordinary fowls. These cells are absent, or rare, in the testis of adult males of ordinary breeds. I suggested, therefore, that the inhibiting hormone of the male sebright is produced by these cells. This hypothesis was, I believe, justified by the evidence, which could not, however, be claimed to be more than circumstantial.

The condition of the sebright is almost unique amongst birds. As a rule the male bird has a more highly developed plumage than the female. One of the rare exceptions to this rule is shown by the phalarope, in which the female is more highly colored than the male. An opportunity to examine the gonads of this bird arose when I was in California in 1920. In the autumn of that year I shot a few birds (*Lobipes lobatus*), adult and young, female and male, and made preparations of their ovaries and testes. In the ovaries the characteristic lutear cells were found, but none could be detected in the testes either of the juvenile or of the adult males. I delayed publishing the result (or lack of result) because I hoped to procure males in the spring, during their migration northward, but so far I have not been successful. However, it is improbable that the spring birds would be different from the autumn migrants with respect to the lutear cells. In a recent number of the *Anatomical Record* (**xxiv.**, No. 6, 1923) Yocum reports, in fact, the absence of lutear cells in the

testis of the phalarope both in the spring and in the autumn migrants.

The absence of the lutear cells in the testis of the male phalarope shows that his failure to develop a plumage as highly colored as that of the female can not be ascribed to such cells. Whether other cells in the testis of these birds produce a hormone that acts as an inhibitor can only be found out by castration. It is, of course, just as likely that the result may be produced in some other way or by other cells. The absence of lutear cells does not count either for or against the view that these cells are the agents in the male sebright that keep his plumage down to that of the female.

I think that too much emphasis is being placed on my hypothesis that the lutear cells in the sebright produce the hormone that suppresses the cock-feathering. It is a matter of secondary importance what cells in the testis of the male sebright produce the hormone. The important result is not the identification of these cells, but rather the demonstration through castration that something is produced by the testis of the male sebright that suppresses the full development of his plumage. This is the result that I should wish to emphasize rather than that the results are brought about by lutear cells. The latter view is hypothetical and, as I have said, is based only on circumstantial evidence. Nevertheless, I still think that the evidence makes this interpretation probable, and that it is not affected by the results here recorded for the phalarope.

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THE VERTEBRAL FORMULA IN THE SAUROPODA

I HAVE had occasion recently to very carefully study the excellent paper by Messrs. Osborn and Mook upon the osteology of *Camarasaurus,* etc., published with a profusion of figures and plates in the *Memoirs* of the American Museum of Natural History, Volume III, Part III. The paper deals with the material representing the Sauropoda collected for Professor E. D. Cope near Canyon City, Colorado, and now possessed by the American Museum of Natural History.

All students of the skeletal structure of the dinosauria have been placed under obligation by this monograph, the difficulty of preparing which can only be realized by the small coterie of

those who have been called upon to make similar studies. Not only are many of the bones heavy and on that account difficult to handle, but in the case of Cope's material they have been more or less crushed and distorted. Unfortunately, as is pointed out by the authors, the original records, if there were any in the possession of Professor Cope, have been lost, and it is impossible at this late day to ascertain what were the exact relations of the different skeletal remains as they originally were found in the quarries.

There is one important point in which I find myself constrained to differ from the conclusions reached by the learned authors of this monograph. Osborn and Mook state on page 291 that "*Apatosaurus* is known to have thirteen cervicals, including the atlas and axis." Upon what this positive statement is founded is not made clear by the writers. It is, I think, open to question. The Carnegie Museum has in its possession a practically complete skeleton, which we have referred to the genus *Apatosaurus,* and this skeleton shows that the cervical vertebræ were *fifteen* in number, including the atlas and axis. Professor Hatcher ascertained beyond the possibility of question that in the genus *Diplodocus* there were fifteen cervical vertebræ, including the atlas and axis. I am strongly of the opinion that *Camarasaurus* possessed fifteen cervical vertebræ, as was the case in the other two genera, to which allusion has just been made, and in which the number of cervical vertebræ has been definitely ascertained. Furthermore, we have in our possession at the Carnegie Museum a cervical vertebra belonging to *Camarasaurus* obtained from one of the same quarries from which Cope's material was taken, and which appears to be a posterior cervical, differing from all of those figured by Osborn and Mook in certain particulars, and in such a way as to suggest that it is one of the posterior cervicals, which they did not possess at the time they were working up the material upon which they founded their memoir. Whether it is the fourteenth or the fifteenth it is hard to say, but it appears to be intermediate between the first dorsal, as figured by Osborn and Mook from more or less fragmentary material, and the last cervical which they have called the thirteenth.[1] In view of the positively ascertained fact that in the

[1] There is probably another missing cervical in the holes which Prof. Cope caused to be made, and which may turn up at some future time when further explorations are made in these localities.

related genera *Apatosaurus* and *Diplodocus* there are fifteen cervicals and the fact that we possess a posterior cervical which was apparently unknown to Osborn and Mook, I am inclined to maintain, until convinced to the contrary, that *Camarasaurus* in all probability possessed fifteen cervical vertebræ.

There is other material, representing other allied genera, which the writer is engaged at the present time in closely studying, as fast as it can be removed from the matrix, which goes to confirm the view which I have just expressed.

The number of vertebræ entering into the composition of the sacrum has been the subject of some diversity of opinion. I am inclined to think that the view expressed by others and accepted by Osborn and Mook that the vertebræ of the sacrum in the Sauropoda are five in number, of which three are primary, preceding which is the dorso-sacral, and succeeding which is the caudo-sacral, is correct. This view is confirmed by all the material at my command, consisting of numerous sacra of sauropods. Osborn and Mook point out very aptly that the degree of coossification between the dorso-sacral and the caudo-sacral and the three primaries varies with age. As a rule the caudo-sacral coossifies more completely with the three primaries and with the ilia than the dorso-sacral. In fact the caudo-sacral, even in young specimens, appears in the different genera to constitute an integral part of the sacrum. So far as is known to me there are very few sacra which have been collected in which the caudo-sacral is not firmly united with the antecedent primary series. It is doubtful whether the sacra in which the three primaries alone are found are in perfect condition. Such a sacrum with only three primaries was figured by Professor O. C. Marsh in the case of *Apatosaurus ajax* and *Atlantosaurus montanus* (*Cf. American Journal of Science*, Vol. XVII, 1897, pl. VI, figs. 1 and 2). My impression is that these sacra are both defective specimens, but the temporary inaccessibility of Marsh's material, owing to the fact that the Peabody Museum is undergoing reconstruction, prevents me at present from confirming this view. These sacra are either defective, or are the sacra of young and immature individuals.

The conclusion which I desire to advance in reference to the vertebral formula in the Sauropod dinosaurs is represented in the following formula, which is advanced as representing the latest generalization upon the subject, largely based upon the material in the possession of the Carnegie Institute.

VERTEBRAL FORMULA IN THE SAUROPODA

Cervicals, including atlas and axis		15
Dorsals		10
Sacrals — Dorso-sacral 1, Primary sacrals 3, Caudo-sacral 1		5
Caudals according to genera		32–82

It is, of course, possible, as more and more material is acquired and studied, that the foregoing formula may be found in one or the other genus of the Sauropoda to show variation in the number of the cervicals. The presumption, however, is strong that the various genera will be found in the end to have the same number of cervicals, dorsals and sacrals. The number of caudals is always likely to reveal more or less generic and even individual variation.

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